Reliability of a New Measure of Motoneuron Excitability

by

Anita Christie

Faculty of Applied Health Sciences
Brock University

A thesis submitted for completion of
Masters of Science Degree

Acknowledgements

First and foremost I would like to thank my advisor, Dr. David Gabriel for introducing me to research, building my confidence, and encouraging me to reach the highest potential. I am truly grateful for all of your help and support and for teaching me so many life lessons in addition to the academic lessons. I will never underestimate the value of scutt work. I would also like to extend my thanks to the entire examining committee Dr. Nota Klentrou, Dr. Diane Stevens and Dr. Jean Boucher for all of their contributions to this document and for the challenging and thought-provoking questions and comments. To Dr. Stevens in particular, thank you for your patience and guidance in an area with which I was completely unfamiliar. Despite all of the complaints it was a pleasure to learn. To my labmates, Kristina, Steve and Deb, thank you for making this an enjoyable journey and for all of your encouragement. Thanks also for reminding me that I need to have a little fun once in a while. The scutt work is all yours now. I would also like to acknowledge my neighbour Greig. Thanks for helping me procrastinate when I needed it and for whipping me into shape, both physically and mentally. To my family, here is the reason I rarely get to see you. Thank you for all of the financial and moral support, it is never underestimated in my mind. It has been an honour to be surrounded by so many wonderful people.

Summary

Objective: This study examined the intraclass reliability of different measures of the excitability of the Hoffmann reflex, derived from stimulus-response curves. The slope of the regression line of the H-reflex stimulus-response curve advocated by Funase et al. (1994) was also compared to the peak of the first derivative of the H-reflex stimulus-response curve (dH/dV_{max}), a new measure introduced in this investigation. A secondary purpose was to explore the possibility of mood as a covariate when measuring excitability of the H-reflex arc.

Methods: The H-reflex amplitude at a stimulus intensity corresponding to 5% of the maximum M-wave (M_{max}) is an established measure that was used as an additional basis of comparison. The H-reflex was elicited in the soleus for 24 subjects (12 males and 12 females) on five separate days. Vibration was applied to the Achilles tendon prior to stimulation to test the sensitivity of the measures on test day four. The means of five evoked potentials at each gradually increasing intensity, from below H-reflex threshold to above M_{max}, were used to create both the H-reflex and M-wave stimulus response curves for each subject across test days. The mood of the subjects was assessed using the Subjective Exercise Experience Scale (SEES) prior to the stimulation protocol each day.

Results: There was a modest decrease in all H-reflex measures from the first to third test day, but it was non-significant (P's>0.05). All measures of the H-reflex exhibited a profound reduction following vibration on test day four, and then returned to baseline levels on test day five (P's<0.05). The intraclass correlation coefficient (ICC) for H-reflex amplitude at 5% of M_{max} was 0.85. The ICC for the slope of the regression line was 0.79 while it was 0.89 for dH/dV_{max}. Maximum M-wave amplitude had an ICC of 0.96 attesting to careful methodological controls. The SEES subscales of fatigue and psychological well-being remained unchanged across the five days. The psychological distress subscale ($P<0.05$), as well as the amplitude of the H-reflex at 5% M_{max} ($P<0.01$) showed a significant cubic trend across the five days. No significant correlation was found between $H_{5\%}$ and psychological distress ($P>0.05$).

Conclusions: The peak of the first derivative of the H-reflex stimulus-response curve (dH/dV_{max}) was shown to have comparable reliability and sensitivity to other more established measures of excitability. Psychological distress and the amplitude of the H-reflex at 5% M_{max} follow similar trends across days, however there was no significant correlation between the two measures.

Significance: The proposed method appears to be a more robust measure of H-reflex excitability than the other methods tested. As such it would be an advantageous method to apply in clinical and investigative settings. Additionally, the results suggest that the relationship between psychological distress and H-reflex amplitude should be investigated further.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Introduction

The Hoffmann Reflex (H-reflex) is a monosynaptic neuromuscular response first described by Hoffmann (1918). It can be evoked in most limb muscles by percutaneous electrical stimulation of the Ia sensory fibres of an associated nerve. Recordings of this reflex are used as a clinical tool to investigate spinal pathways and to quantify the level of excitability of the motoneuron pool (Kamen & Caldwell, 1996). There are several measures that can be extracted from an H-reflex recording to provide an estimate of excitability. However, we are concerned with the reliability of these measures. A reliable measure of excitability provides confidence that any changes reflect physiological changes, not variability in the measurement technique. The reliability of a measure can have important implications in both experimental planning and in clinical settings. A higher reliability translates to lower error and higher statistical power. Information about the reliability of a measure is therefore important in sample size estimation. In clinical settings, a more reliable measure increases confidence that any observed changes truly reflect changes to the biophysical properties of the system under investigation.

Traditionally, the characteristics of the H-reflex used to make clinical assessments are the maximum amplitude of the response, or the amplitude of the response at certain percentages of the maximum amplitude of the motor response (M_{max}). The maximum amplitude of the H-reflex has been demonstrated to be reliable across days (Hopkins et al, 2000). However, the literature focusing on the reliability of each of these measures is limited, despite their wide-spread use. None of these measures is without limitations that interfere with the estimate of excitability of the reflex arc, which could affect their reliability. Funase et al. (1994), therefore, have suggested an alternate method of using H-reflex recordings to assess neuromuscular activity, which attempts to avoid these limitations.

The method proposed by Funase et al. (1994) suggests that the slope of the regression line of the H-reflex stimulus-response curve (S-R curve), calculated below the threshold of the M-response can be used as a measure of excitability of the reflex arc. In theory, changes in neuromuscular excitability alter the slope of the stimulus-response curve. An increase in excitability would result in an increase in the peak-to-peak amplitude of the response, which would lead to an overall shift of the stimulus-response curve to the left. The opposite would be true for a decrease in excitability. It is suggested that because this method is free from any collision effects, it is an effective method of evaluating excitability (Funase et al., 1994). More recently, this method has been used clinically in assessments of recovery of hemiplegic patients (Higashi et al., 2001). However, the characteristics and the reliability of this method have not been investigated, and it is not without its own limitations.

Funase et al. (1994) calculated the slope using a linear regression, which was a least-squares fit to the ascending limb of the stimulus-response (S-R) curve. The ascending limb of the curve, however, is sigmoidal in shape and is poorly approximated by a linear fit. Since the slope of the regression line provides a rate of change in the curve, this study explores the first derivative of the H-reflex S-R curve as an alternate, perhaps an even better, method of assessment. The peak of the first derivative of the H-reflex S-R curve takes into account all of the points on the ascending limb of the curve and provides an indication of the maximal rate of change of excitability of the reflex arc. However, the measurement characteristics and the reliability of this proposed method of assessment are also unknown.

There are several factors that are known to affect the reliability of an H-reflex recording. These factors include, but are not limited to, the position of the subject (Hopkins et al., 2000; Al-Jawayed et al., 1999), the placement of electrodes (Delagi & Perotto, 1980), the duration of the stimulus (Williams et al., 1992) and the interval between stimuli (Pierrot-Deseilligny & Mazevet, 2000). One factor that may also be related to changes in reflex excitability is the mood, of the subjects. Mood is how an individual feels at any given moment. It is affected by environmental stimuli and can fluctuate readily (Silva & Weinberg, 1982). The possible relationship between mood and spinal excitability has not been investigated. It has been suggested that anxiety associated with the anticipation of a noxious stimulus can affect force production in interpolated twitch studies (Behm & Button, 2002), where the nerve is electrically stimulated during a maximum voluntary contraction. The construct of mood, however, has never been measured in such a study.

The primary purpose of this research is to investigate the reliability of estimating excitability of the reflex arc using selected criterion measures of the H-reflex. These measures include the following: (1) calculating the slope of the regression line of the stimulus-response curve of the H-reflex, (2) calculating the peak of the first derivative of the stimulus-response curve, and (3) the traditional method of calculating the maximum amplitude of the H-reflex response and the amplitude at 5% M_{max}. Vibration will also be used to test the sensitivity of each measure to physiological changes.

A secondary purpose of this study, therefore, was to investigate the possibility that mood is a covariate in measures of the excitability of the H-reflex arc. On each of the five test days, subjects were asked to fill out a Subjective Exercise Experience Scale (SEES). This is a twelve-item, self-report measurement developed to assess mood following an exercise bout (McAuley &

Courneya, 1994). It consists of questions aimed at investigating three subscales of psychological state: Psychological Well-being, Psychological Distress and Fatigue. Subjects were asked to respond to each of the questions on a seven-point Likert Scale ranging from "not at all" (1) to "very much so" (7).

Investigation of the reliability of the measures and of the possibility of psychological state as a covariate was accomplished using a repeated-measures design. Twenty-four subjects participated in a total of five test sessions. During each session the H-reflex was elicited by stimulation of the tibial nerve and the resulting electromyographic (EMG) activity was recorded from the soleus. The stimulus intensity was gradually increased from below the threshold of the H-reflex to above the maximum M-wave, with five stimuli being delivered at each intensity. The mean peak-to-peak amplitude of the responses was used to generate a stimulus-response curve for the H-reflex and the M-wave on each day. Each of the criterion measures were drawn from these stimulus-response curves across days to determine reliability. The first three test days were used to establish a baseline for each of the measures. On test day four vibration was applied to the Achilles tendon immediately prior to nerve stimulation to test the sensitivity of the measures. Day five was then used to ensure that the measures return to baseline.

A repeated measures analysis of variance and calculation of the intraclass correlation coefficient were used to determine the reliability of each of the H-reflex measures. A repeated measures analysis of variance with an orthogonal polynomial breakdown was used for statistical trend testing for means of H-reflex measures and the three subscales of the SEES across days. This provides important implications in future studies using the H-reflex, where, perhaps mood would be a useful covariate in neuromuscular studies using the H-reflex.

1.1 Assumptions

The following assumptions were made in the present investigation:

1. Surface electromyography recordings of the H-reflex reflect excitability of the H-reflex arc
2. The slope of the regression line of the H-reflex stimulus-response curve is a measure of the rate of change in motoneuron excitability as a function of increased input to the Ia sensory fibers.
3. The first derivative of the H-reflex stimulus-response curve is a measure of the maximum rate of change in H-reflex arc excitability as a function of increased input to the Ia sensory fibers.
4. The maximum amplitude of the M-response represents the stimulus intensity at which all motor units are being recruited.
5. Mood is a construct that can be measured by self-report.
6. Mood can be accurately measured by the Subjective Exercise Experience Scale.
7. Subjects responded to all of the questions on the Subjective Exercise Experience Scale honestly and accurately.

1.2 Delimitations

1. This study included only college-aged subjects, free of neurological disorders.
2. Only the right leg was investigated.
3. Only the soleus muscle was investigated.
4. Mood was measured only with the Subjective Exercise Experience Scale.

1.3 Limitations

1. Since only college-aged subjects were tested the results of this investigation may not apply to populations of a different age group.
2. Since only the right leg was investigated the results may not apply to the left side of the body. Side-to-side differences may exist.
3. Since only one muscle in the lower leg (the soleus) was investigated, the results may not apply to muscles in other limbs or to other muscles in the lower leg.
4. Since this study used only one method of investigating mood, the prediction of mood through alternative measures may not be applicable to these results.

1.4 Definitions

Electromyography (EMG) – A technique used to record electrical signals within skeletal muscle.

Excitability – how close a cell is to its firing threshold.

H-Reflex – An electrically-evoked stretch reflex first described by P. Hoffmann (1918). A monosynaptic neuromuscular response, readily measured in most limb muscles.

Intraclass Correlation Coefficient (ICC) – A comprehensive method of assessing reliability using values from an analysis of variance. Provides a reliability coefficient for two or more measurements on the same element.

Motoneuron Pool – A group of motoneuron cell bodies within the spinal cord.

Motor Unit – A motoneuron and all of the muscle fibers it innervates.

Monosynaptic Reflex – An involuntary reaction to a stimulus involving a single synaptic connection.

M-Response – A direct motor response of a muscle evoked by exciting motor fibers through electrical stimulation of a nerve.

Mood – A relatively stable subjective feeling state. A multidimensional construct anchored by positive and negative poles.

Reliability – Repeated measurements on the same person results in similar scores during baseline conditions.

Subjective Exercise Experience Scale (SEES) – A self-report questionnaire designed to give a multidimensional assessment of mood. Addresses three subscales: Psychological Well-being, Fatigue and Psychological Distress.

Chapter 2: Review of Literature

2.1 Electromyography

Electromyography (EMG) is the study of muscular activity through analysis of the electrical signals associated with voluntary and involuntary muscle contractions (Basmajian & DeLuca, 1985). Two methods of obtaining EMG recordings are through the use of indwelling or surface electrodes. Indwelling recordings are accomplished by inserting fine wire electrodes directly into the muscle fibers. This method is advantageous in that it allows for the observation of activity within a single motor unit (Gabriel et al., 2001). However, it is debatable whether these recordings reflect the behaviour of the whole muscle (Rau & Disselhort-Klung, 1997). Surface EMG involves the recording of such electrical activity through electrodes placed on the surface of the skin over a muscle. This method is advantageous in that the recordings represent activity within the muscle as a whole (Rau & Disselhort-Klung, 1997).

A motor unit includes a motor axon and all of the muscle fibres it innervates (Figure 1). The action potential of a motor unit is the result of spatial and temporal summation of action potentials in all of the fibres comprising a single motor (Basmajian & DeLuca, 1985). The surface EMG signal, therefore, reflects the summation of action potentials in all motor units within the pickup area of the surface electrode (Figure 2). Muscle fibres of several different motor units interdigitate throughout the muscle. Thus, the area under an electrode can include fibres from as many as 20 to 50 motor units and an electrode will always pick up signals from more than one motor unit (Basmajian & DeLuca, 1985). As the general activity level within the muscle increases, so too will the number of summated motor unit action potentials detected by the recording electrode (Kamen & Caldwell, 1996). The frequency of the signal detected by the

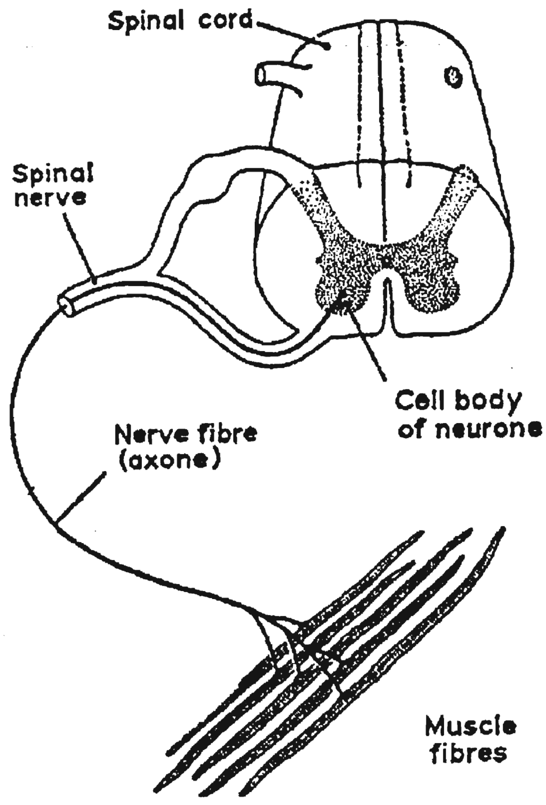

Figure 1. A motor unit is comprised of the motor neuron and all of the muscle fibers it innervates. Basmajian, J.V., and DeLuca, C.J. (1985). *Muscles Alive: Their function revealed by electromyography* (5th ed.). Baltimore, MD: Williams & Wilkins, Figure 1.7, page 12.

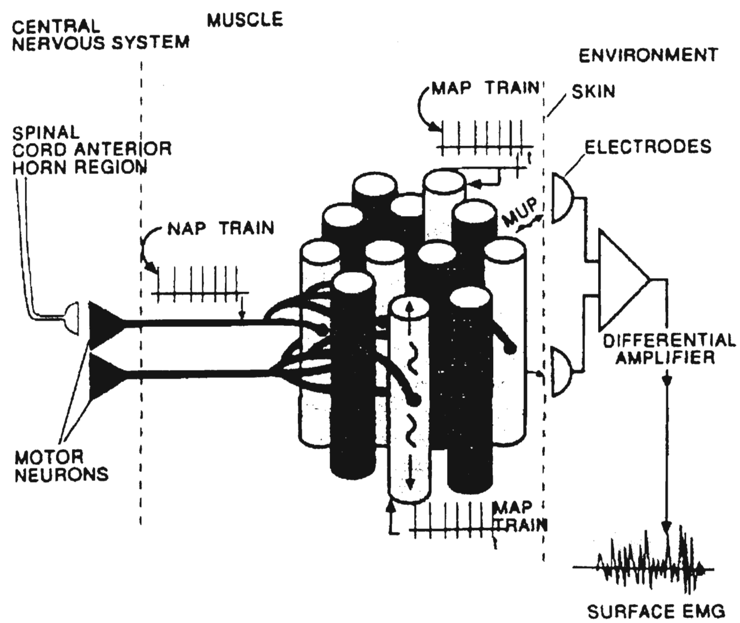

Figure 2. Origin of the EMG signal. Chaffin, D.B., Andersson, G.B.J., and Martin, B. (1999). *Occupational biomechanics* (3rd ed.). New York, NY: Wiley & Sons, Inc., Figure 5.6, page 147.

electrode corresponds to the number of active motor units, as well as the firing rates within each active unit (Kamen & Caldwell, 1996). The amplitude of an EMG recording is normally distributed, ranging from 0 to 10 millivolts (Basmajian & DeLuca, 1996). The detection area of an EMG surface electrode is always the same, in terms of depth and breadth, irrespective of the size of the muscle (Kamen & Caldwell, 1996). Therefore, recordings from smaller muscles include a greater proportion of the fibres within the muscle, compared to recordings from larger muscles (Kamen & Caldwell, 1996).

There are several factors that can cause variation in the characteristics of the EMG signal recorded from the surface of the skin, in addition to the physiological processes (Kamen & Caldwell, 1996). Figure 3 illustrates that surface EMG recordings of electrical activity in the muscle are dependent upon the following factors: the thickness of adipose tissue between the muscle fiber and the electrode, the diameter of the muscle fibres and the filtering capacity of the electrode (Basmajian & DeLuca, 1985). Adipose tissue acts as a low-pass filter (Soderberg, 1992). Therefore, in recordings of muscle activity, a large volume of adipose tissue between the muscle and the recording electrode will result in a predominance of low frequency signals. High frequency signals, however, will be filtered out and will be reduced. The filtering capacity of the electrode affects the signal in a manner similar to adipose tissue. Depending on the electrode, high or low frequency signals can be filtered out and are therefore minimized.

A motor unit is a motoneuron and all of the muscle fibers it innervates. Small diameter motoneurons have a lower threshold for activation than motoneurons with a larger diameter. The recruitment of motor units occurs according to the size principle (Henneman, 1957), with smaller motoneurons being recruited before larger ones. The number of active motor units contributes to the amount of force the muscle produces and to the amplitude of the EMG signal. The frequency at which each motor unit is firing will also contribute to force production and the amplitude of the EMG recording (Lamb et al., 1992). The type of muscle fibers that the motor neuron innervates can also affect the EMG signal. Within the muscle there are two types of fibers innervated by the motoneurons: slow-twitch oxidative (type I) and fast twitch (type II). Fast-twitch muscle fibres have a larger diameter than slow-twitch fibers. The activation of these different fiber types contribute to the EMG signal. The soleus for example, is comprised mainly of slow-twitch fibers, with each motoneuron activating approximately 1700 muscle fibers (Bonnet et al., 1981). The recruitment of small diameter, slow-twitch fibers results in recordings of lower frequencies, while large diameter, fast- twitch fibers contribute to the higher frequency component of the recording (Gabriel et al., 2000). Additionally, fast-twitch fibers produce greater amplitude action potentials than slow-twitch fibers (Kamen & Caldwell, 1996), resulting in greater peak-to-peak amplitudes in the EMG recording.

The muscles within the limb are sometimes very close together and work together to produce a force or movement. This can pose a problem because the signals from adjacent muscles are picked up by the recording electrodes. This is called cross-talk (Basmajian & DeLuca, 1985) and can interfere with obtaining a true representation of the activity within the muscle of interest. Cross-talk is minimized by placement of the electrode on the muscle belly, and demonstrating that the primary muscle is active while adjacent muscles are silent. It is often monitored by recording from several muscles simultaneously to ensure only the muscle of interest is activated. Additionally, with high force contractions there is often movement of the muscle and/or the wires of the recording electrodes. Movement of either the limb or the wires will be picked up as an electrical signal by the electrodes (Basmajian & DeLuca, 1985), termed movement artifact, which will also interfere with obtaining a true recording of muscle activity.

To avoid this movement artifact, both the electrode wires and the limb of the patient should be secured.

Another factor that can affect the accuracy of the EMG signal is the signal-to-noise ratio (Basmajian & DeLuca, 1985). Noise is generated by any electrical source that operates at 60 cycles per second. To decrease the noise in a recording, thereby increasing the signal-to-noise ratio, a differential recording configuration is used (Basmajian & DeLuca, 1985). This method involves the use of two recording electrodes placed on the surface of the skin at a fixed distance from one another. The electrical signal within the muscle, as well as noise is detected at both sites. Signals that originate at some distance from the electrodes (i.e. the 60 Hz noise from electrical devices) will appear as a common signal to the two electrodes and will be cancelled by differential recording. Any signal that originates in the immediate vicinity of the electrodes (i.e. action potentials in the muscle fibers), however, will appear at a different time between the two electrodes. The different signals detected at the two electrodes will be subtracted and amplified as shown in Figure 4 (Basmajian & DeLuca, 1985).

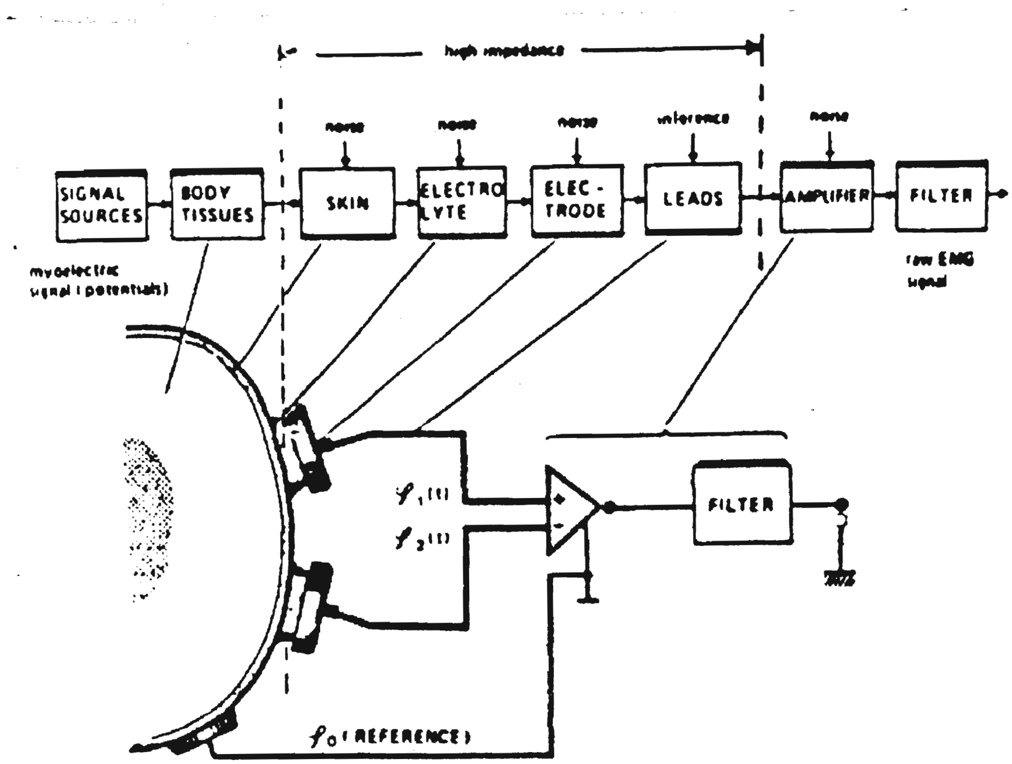

Figure 3. Factors affecting an EMG recording. Soderberg, G. Selected topics in surface electromyography for use in the occupational setting: Expert perspectives. DHHS (NIOSH), Publication No. 91-100, Washington DC, NIOSH, 1992.

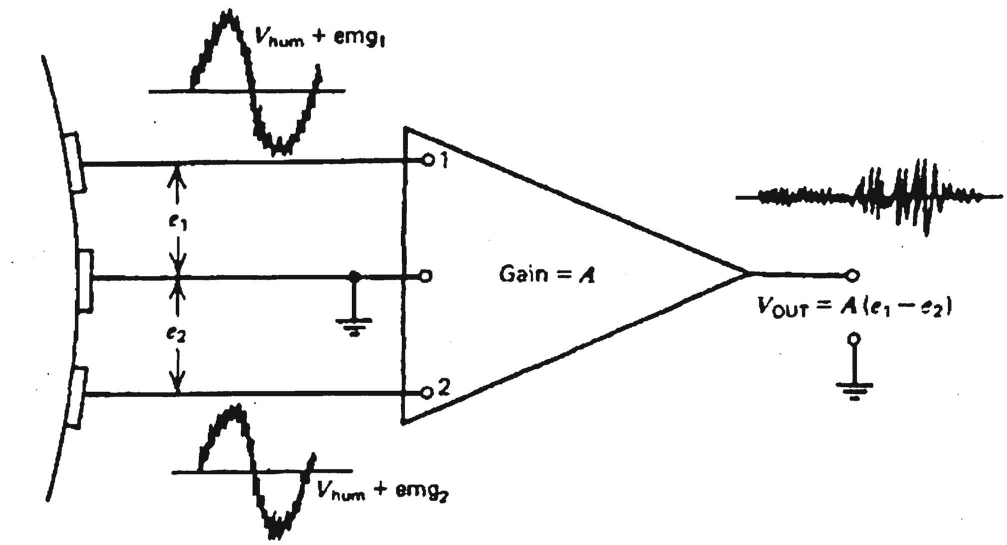

Figure 4. Differential recording technique. Winter, D.A. (1990). *Biomechanics and motor control of human movement* (2nd ed.). New York, NY: Wiley & Sons, Inc., Figure 8.8, page 202.

2.2 H-Reflex Circuitry

2.2.1 The Monosynaptic Reflex. Hoffmann (1918) first demonstrated the monosynaptic H-reflex (taken from Zehr, 2002). The reflex involves three components, as illustrated in Figure 5A: (1) the sensory afferent pathway comprised of spindle receptors innervated by large diameter Ia sensory fibers, (2) synapses within the ventral horn of the spinal cord, and (3) the efferent pathway formed by alpha motoneurons that innervate muscle fibres (Preston & Shapiro, 1998; Bonnet et al., 1981).

Using a low intensity stimulus applied to a peripheral nerve, it is possible to selectively activate the Ia sensory fibres of that nerve (Preston & Shapiro, 1998). The synchronous stimulation of any number of the Ia sensory fibers will result in the depolarization of many or all of the motoneurons innervating the associated muscle (Bonnet et al., 1981). If the stimulus intensity is strong enough to reach the threshold of the Ia fibers, action potentials will be generated in these fibers. These action potentials travel orthodromically, along the Ia afferent fibers to the ventral horn of the spinal cord, where they synapse with the α-motoneurons. If the sensory signal is strong enough, it will depolarize the neurons. The depolarization of these motoneurons generates action potentials, which also travel orthodromically along the axon of the motoneuron to the muscle (Preston & Shapiro, 1998).

The recorded response of the muscle to selective stimulation of the Ia fibers is the H-reflex, depicted in Figure 5A. The recorded H-reflex is triphasic and includes the simultaneous activity of several motor units (Bonnet et al., 1981). There is a relationship between the amplitude of the recorded H-reflex and the number of active motor units (Bonnet et al., 1981).

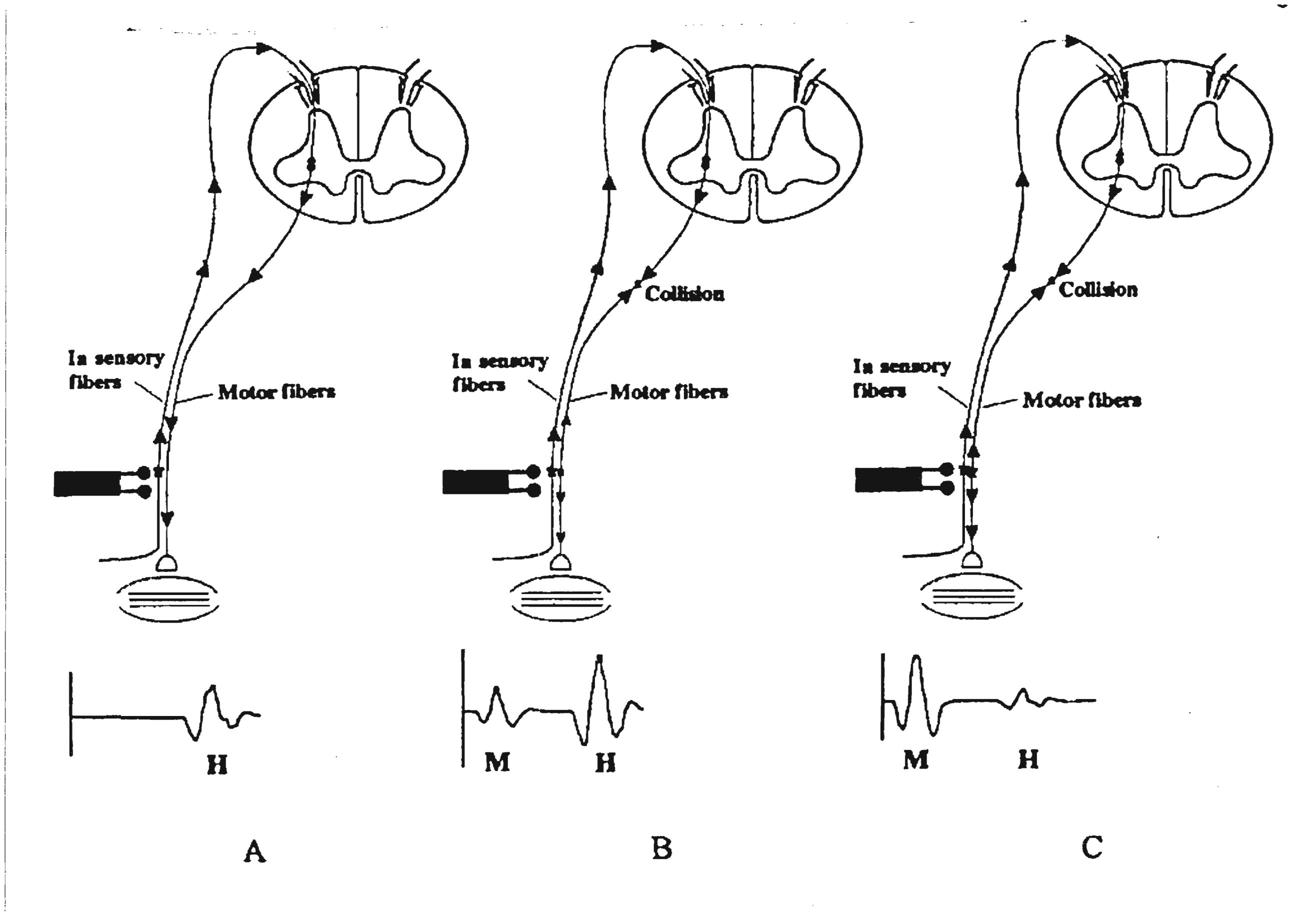

Figure 5. H-reflex circuitry. Preston, D.C., and Shapiro, B.E., (1998). *Electromyography and neuromuscular disorders: Clinical-electrophysiological correlations.* Boston, MA: Butterworth-Heineman, Figure 4.8, page 52.

The amplitude of the H-reflex increases and the latency decreases with slowly increasing stimulus intensity (Preston & Shapiro, 1998). A further increase in stimulus intensity will result in the direct stimulation of both the Ia sensory afferents and the motor axons. When this occurs a direct motor potential (M-wave) is generated by action potentials that travel orthodromically from the point of stimulation on the motoneuron along the axon to the muscle. The latency of this response is smaller than that of the H-reflex, as the pathway from the point of stimulation to the muscle is shorter (Figure 5B).

When the stimulus intensity is high enough to directly stimulate the motoneurons, motor action potentials not only travel to the muscle, but also travel from the point of stimulation, antidromically, toward the ventral horn of the spinal cord (Preston & Shapiro, 1998). These antidromic motor potentials collide with the orthodromic sensory potentials. Collision is a result of the properties of the membrane channels. Briefly, during an action potential the Na^+ channels on the membrane surface open and there is an influx of Na^+. These channels are then inactivated and K^+ channels are activated. While the K^+ channels are active, the membrane is in a refractory period, meaning that no stimulus will be strong enough to depolarize the membrane. The membrane of the motoneuron will be in a refractory period as a result of the antidromic motor potentials. The orthodromic action potentials, resulting from synaptic transmission in the ventral horn, will be inhibited as they reach these areas of the motoneuron membrane, which cannot be depolarized. As a result, the sensory signal is impeded from reaching the muscle and, consequently, a decrease in the amplitude of the H-reflex is observed. The flow of information at the synapse in the ventral horn is unidirectional, from the sensory fibers to the motor fibers. Therefore, the collision occurs in the motor fiber, as the antidromic action potentials in the motoneuron cannot cause depolarization of the Ia sensory fiber.

The amount of collision is dependent on the strength of the stimulus and is a function of the diameter of the sensory and motor fibres and of the motor unit recruitment patterns of electrical and sensory stimulation. Within the motoneuron pool the motor fibres are recruited according to Henneman's size principle, from smallest to largest (Henneman, 1957). Larger diameter fibres however, have a lower axial resistance and a higher capacitance. Electrical stimulation, therefore recruits fibres in the reverse order, from largest to smallest. When the stimulus intensity is such that both the H-reflex and M-wave are observed, the largest diameter sensory and largest diameter motor fibres are recruited by the electrical stimulation. However, at the motoneuron pool, the large-diameter sensory fibres activate the smaller diameter motor fibres. Therefore, at low to moderate stimulus intensities, different motor fibres are activated by electrical stimulation and stimulation of the sensory fibres. Thus, the sensory signal is still able to reach the muscle, even if there is some overlap in the recruited motor fibres.

As stimulus intensity increases more small diameter motor fibres are recruited by the electrical stimulation, resulting in greater overlap in the motor fibres activated by the sensory and electrical stimulation. This results in an increase in collision as more motor fibres are in a refractory period from the antidromic action potentials. This increase in collision results in the signal from the Ia fibers being completely lost in the motor fibers. The H-reflex then disappears and the amplitude of the M-wave increases until the maximal response is reached (Figure 5C) at which time, all motor units are being recruited.

2.2.2 The M-Response. To generate an M-wave, the peripheral nerve must be electrically stimulated at a sufficient intensity to activate small-diameter motor axons (Pierrot-Deseilligny & Mazevet, 2000). Although there is some overlap in the diameter of Ia sensory fibres (12-20μm) and α-motoneurons (8-16μm), sensory fibres generally have a larger diameter.

Because of this larger diameter, Ia sensory fibres have a lower axial resistance, more mylenation and greater capacitance than α-motoneurons (Schalow & Zach, 1994). All of these characteristics contribute to the lower threshold of the sensory fibres. It follows then that the M-wave, activating small diameter motor fibres, is evoked at higher stimulus intensities than those that evoke the H-reflex which activate large diameter sensory fibres (Pierrot-Deseilligny & Mazevet, 2000). The amplitude of the M-wave gradually increases with increasing stimulus intensity, until the maximum amplitude of the response is reached. Reaching the maximum amplitude M-wave indicates that all motor axons innervating the muscle have been recruited (Herbert & Boucher, 1998).

It is important to measure the maximal M-wave during H-reflex testing (Pierrot-Deseilligny & Mazevet, 2000; Herbert & Boucher, 1998). The M-wave is the result of direct stimulation of the alpha motoneurons. It is a stable response that is not affected by other inputs or by changes at the level of the spinal cord. If the M-wave is stable, any changes observed in the H-reflex must be neural in origin, rather than due to changes in experimental conditions or other factors that affect the muscle itself.

2.2.3 The Recording Site. H-reflexes can be reliably recorded from any muscle in the limbs with an innervating nerve that is accessible for electrical stimulation (Miller et al., 1995; Pierrot-Deseillgny & Mazevet, 2000). Limb muscles are very useful because there is enough distance between these muscles and the spinal cord to allow the M-response and the H-reflex to appear as two distinct waves (Miller et al., 1995). While the soleus muscle of the lower leg is the most widely studied muscle in H-reflex investigations, reliable recordings can be obtained from other muscles as well. The diameters of the Ia fibers of the soleus, gastrocnemius and the flexor carpi radialis are larger than the diameter of the motor axons, making it easy to generate an H-reflex in these muscles (Pierrot-Deseilligny & Mazevet, 2000). It has also been demonstrated that the soleus and the lateral and medial gastrocnemius, are sensitive to changes in neuromuscular activity (Herbert & Boucher, 1998).

2.2.4 Inhibition. The H-reflex is influenced by any change in the excitability of any of the components of the monosynaptic reflex. Such changes can include: the activity of gamma motoneurons controlling the stretch sensitivity of the muscle spindles; the excitability of the Ia afferent fibers and inhibitory pathways controlling the transmission capacity of the Ia fibers (Bonnet et al., 1981).

The Ia afferents are constantly under the influence of presynaptic control. Which alters their ability to transmit signals (Kernell & Hultborn, 1990; Morita et al., 1998; Zehr, 2002). When stimulating the nerve electrically, it is possible to activate inhibitory pathways, which can decrease the amplitude of the H-reflex response (Earles et al., 2002). Two disynaptic pathways exist which may be responsible for this inhibition. These pathways include Ib inhibitory interneurons and primary afferent depolarization (PAD) interneurons (Pierrot-Deseilligny & Mazevet, 2000).

Motoneurons activated early during the H-reflex can recruit the Renshaw cells, a recurrent inhibitory influence. Both Renshaw cells and the Ia afferents converge on Ib inhibitory interneurons (Marchand-Pauvert et al., 2002). Electrically evoked EPSPs contributing to the H-reflex have a long run time. This allows enough time for disynaptic inhibitory post-synaptic potentials (IPSPs) to de-recruit the motoneurons that contribute late to the H-reflex, thus decreasing the amplitude of the response (Pierrot-Deseilligny & Mazevet, 2000). Primary afferent depolarization of Ia fibers is accompanied by presynaptic inhibition of the Ia terminals. This presynaptic inhibition is the result of the activation of axo-axonal GABA-ergic synapses.

The inhibitory signal is communicated to the Ia afferents by PAD interneurons (Pierrot-Deseilligny & Mazevet, 2000). These inhibitory influences can change significantly within an individual and must be considered when assessing changes in the amplitude of the H-reflex.

Investigation of presynaptic inhibition is accomplished through methodologically altering the level of inhibitory input in the motoneuron pool. The H-reflex response can be facilitated by applying a sub-threshold electrical stimulation to the nerve prior to evoking the H-reflex (Zehr, 2002). In the case of the soleus, this can be accomplished by stimulating either the tibial nerve or the femoral nerve (Hultborn et al., 1987; Morin et al., 1984). The afferent volley evoked by sub-threshold stimulation of either of these nerves will increase the discharge of the Ia afferents of the tibial nerve, effectively decreasing presynaptic inhibition and resulting in facilitation of the H-reflex (Zehr, 2002; Morin et al., 1984).

The more common method of investigating the level of presynaptic inhibition, however, is to increase the level of inhibition in the motoneuron pool. This is accomplished by applying a conditioning stimulus to a nerve supplying an antagonistic muscle, just prior to applying a test stimulus to the nerve supplying the muscle of interest. In the case of the soleus, this is accomplished by applying a stimulation to the common peroneal nerve, supplying the tibialis anterior, at an intensity of 1-1.5 times motor threshold followed by stimulation of the tibial nerve (Zehr, 2002; Aymard et al., 2000; Morin et al., 1984). Such inhibition increases with increasing frequency of the stimulation, but has been observed at frequencies as low at 0.5 Hz (Kohn et al., 1997) and appears to have the greatest inhibitory effects when the inter-stimulus interval is between 150-500 ms (Amyard et al., 2000; Kagamihara et al., 1998; Kohn et al., 1997). It has been suggested that the difference in the amplitude of the H-reflex with and without imposed inhibition reflects the level of inhibition that inherently exists at the motoneuron pool and is sometimes used as an indication of excitability (Zehr, 2002).

While it is acknowledged that such inhibitory inputs may affect the amplitude of the H-reflex recordings, these inputs were not investigated in the present study. The primary focus of this investigation was to determine the characteristics of a newly applied (Funase et al., 1994; Higashi et al., 2001) and a newly proposed method of assessing the H-reflex produce similar results consistently enough to be useful clinically. The reliability of the estimation of inhibition is a separate issue that should also be investigated.

2.3 Vibration

When investigating a method of assessment, it is beneficial to examine the sensitivity of the method in order to better determine its characteristics. One way of investigating sensitivity is to perturb the system under investigation and determine how the perturbation affects the measurement properties. Perturbation of the H-reflex "system" can be accomplished through facilitation or inhibition of the reflex. Methods of facilitating the H-reflex include having the subjects perform such activities as the Jendrasic maneuver or activating corticospinal pathways supplying the recording muscle, through mental imagery (Bonnet et al., 1997). However, these methods are difficult, if not impossible to control for, and to ensure consistency between subjects and consistency within subjects over several trials. Common methods of inhibition of the H-reflex include a double stimulation protocol (Zehr, 2002; Aymard et al., 2000; Morin et al., 1984), or vibration of the muscle tendon.

Vibration of the Achilles tendon results in what is known as the vibration paradox, or two opposing reflex responses in the soleus. First, such vibration results in an increase in the level of muscle activation (Desmedt & Godaux, 1978). This is called the tonic vibration reflex and is characterized by a tonic contraction of the muscle. Second, vibration of the Achilles tendon has been shown to produce a large decrease in the amplitude of the soleus H-reflex (Zehr, 2002; Calvin-Figuiere et al., 1999; Herbert & Boucher, 1998; Hilgevoord et al., 1996). The primary afferent muscle spindle fibres (Ia fibres) are the most sensitive of any proprioceptive fibres to vibration and the effect increases with increased frequency of the vibration (Roll, 1989). While it is known that the vibration selectively activates the primary Ia spindle afferent fibres, the two independent and seemingly opposing effects of such proprioceptive input is not fully understood (Desmedt & Godaux, 1978). Vibration of the Achilles tendon is used as a tool to cause depression of the H-reflex. Although the exact mechanism of this depression is not understood, it has been suggested that vibration of the tendon causes an increase in presynaptic inhibition of the Ia motoneurons supplying the agonist muscle (Zehr, 2002, Calvin-Figuiere et al., 1999). Despite the lack of a full understanding of the mechanisms by which tendon vibration inhibits the H-reflex, it is a useful tool for manipulating the physiological properties of the H-reflex to test the sensitivity of different measures of motoneuron excitability.

2.4 Traditional Methods of Assessing the H-Reflex

The assessment of motoneuron excitability using the H-reflex is typically accomplished through investigation of the amplitude of the response. These methods include determining the maximum amplitude of the H-reflex, or the amplitude at a certain percentage of the maximum amplitude of the M-response (Hugon, 1970). Additionally, measures of the H-reflex are often standardized by expressing them as a ratio of the M-response. However, it is thought that each of these characteristics has important limitations in estimating neuromuscular excitability (Funase et al., 1994).

2.4.1 Amplitude of the H-Reflex. It is assumed that the maximum amplitude of the H-reflex is a representation of the maximum number of active motoneurons in the motoneuron pool, in response to electrical stimulation of the Ia fibers (Funase et al., 1994). However, the maximum amplitude of the H-reflex does not occur until after the appearance of the M-wave, and is reduced by the collision occurring between the orthodromic sensory signal and the antidromic motor potential. Maximum H-reflex amplitude is therefore not a true representation of the maximal activity in the motoneuron pool because there is more activity than reaches and is recorded from the muscle (Funase et al., 1994). The same limitation applies to calculating the amplitude of the H-reflex at a certain percentage of the maximum amplitude of the M-response. The higher the percentage of the maximum M-response that is used, the greater the collision in the motoneuron. Such measures of H-reflex excitability, therefore, are not true representations of the activity in the motoneuron pool (Funase et al., 1994).

2.4.2 Ratio Measures. The M-response is a direct motor response. As such, it is not affected by physiological changes at the level of the spinal cord. The M-response, therefore, should not change if the test conditions are consistent. Thus, measures of the M-response are used to standardize measures of the H-reflex. In theory, any variability in the H-reflex due to experimental conditions will also be present in the M-response. Expressing the measure of the H-reflex as a ratio of the same measure of the M-response, in effect, cancels out the experimental error (Funase et al., 1994). However, while the absence of changes in the maximum amplitude of the M-response is an ideal condition, changes in this measure have been observed. Crone et al (1999) have reported decreases in maximum amplitude of the M-response as large as 35.6% in healthy subjects. While these decreases are typically observed when the M-response is evoked several times in one session, such changes may limit the accuracy of these measures.

2.5 New Methods of Assessing the H-Reflex

Despite the relative simplicity of evoking and recording H-reflexes, a valid interpretation of the recordings requires proper analysis (Pierrot-Deseilligny & Mazevet, 2000). One method of analysis involves the H-reflex and M-wave stimulus-response curves (Bonnet et al., 1981). A standard stimulus-response curve is illustrated in Figure 6.

2.5.1 Slope of the Regression Line of the H-Reflex Stimulus-Response Curve. Funase et al. (1994) have proposed that the slope of the H-reflex stimulus-response curve is a measure of neuromuscular excitability with few limitations. The slope is used to represent "reflex gain", or the ratio of the number of motoneurons recruited (amplitude) to the intensity of the stimulus. It is important to emphasize that the stimulus intensity must be below the motor threshold to eliminate the possibility of collision in reducing H-reflex amplitude (Funase et al., 1994). The slope of the H-reflex S-R curve has a high positive correlation (r=.823) with the maximum H-reflex amplitude, and a high negative correlation (r=-.714) with the H-reflex threshold (Funase et al., 1994). Therefore, changes in amplitude and threshold are accompanied by changes in the slope of the S-R curve. This provides further support for the idea that the slope of the H-reflex S-R curve is indicative of changes in reflex arc excitability.

The appearance of the stimulus-response curves for the H-reflex and the M-wave can vary widely between individuals (Funase et al., 1994). However, stimulus-response curves derived over several sessions with the same individual are quite similar (Fuanse et al., 1994). Although the amplitude of the H-reflex has proven to be a reliable measure (Williams et al., 1992), it is not known if such reliability extends to the slope of the H-reflex stimulus-response curve.

2.5.2 First Derivative of the Stimulus-Response Curve. One limitation of the slope of the H-reflex stimulus-response curve lies in how it is determined. Funase et al. (1994) used a linear regression analysis to fit a straight line to the ascending limb of the stimulus-response curve. However, the ascending limb of the curve is sigmoidal in shape and is therefore poorly approximated by a linear fit, which calls into question the validity of this measure. Since the slope is a measure of the rate of change, this study explores the first derivative of the H-reflex recruitment curve as a potential alternative. It has been demonstrated that neurophysiological properties of motor unit recruitment patterns can be accurately and reliably represented by mathematical analysis of the frequency distributions (Yaar, 1999). Curve fitting techniques, therefore can be applied to H-reflex data to generate a normative S-R curve which reflects muscle activity. The peak of the first derivative can then be calculated, providing a true representation of the rate of change in excitability. The first derivative of the H-reflex recruitment curve offers the advantages of utilizing all the points on the curve and accounting for the original sigmoid shape. Thus this method appears to more accurately reflect the biophysical properties of the membrane excitability under consideration.

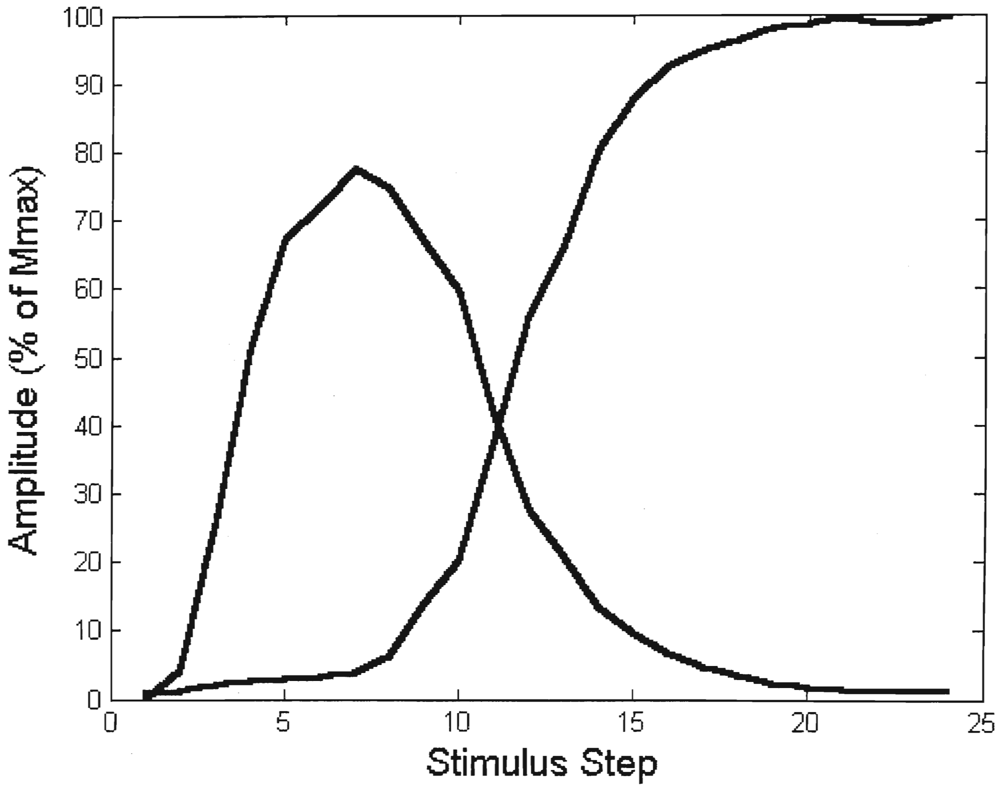

Figure 6. Sample stimulus-response curve of the H-reflex and the M-response.

2.6 Reliability

2.6.1 Inter- and Intra- individual Differences in the H-reflex. Funase et al. (1994) suggest that the H-reflex is unique to individuals and should therefore be consistent within individuals if testing conditions are constant. For this reason, the H-reflex is a widely used tool for investigating the excitability of the reflex arc, both in clinical and research settings. However, the literature documenting the reliability of the H-reflex is scant. The majority of H-reflex reliability data that exist, focus on the reliability of the maximum peak-to-peak amplitude of the response. In general, these investigations report that the H-reflex is a very robust measure of excitability. It has been shown that H-reflex recordings from the soleus are very reliable within a subject across trials, with Pearson r-values greater than 0.95 (Handcock et al., 2001; Williams et al., 1992). It has also been demonstrated that the H-reflex evoked within an individual becomes more consistent with repeated trials (Williams et al., 1992). Furthermore, the maximum peak-to-peak amplitude within individuals across test days has also been found to be reliable with ICC(3,1) values as high as 0.99 reported for the triceps surae (Handcock et al., 2001; Morelli et al., 1990). While intra-individual differences are small, inter-individual variability is quite high for both the H-reflex and the M-response (Williams et al., 1992). The primary cause of this inter-individual variation is differences in excitability of the reflex arc between individuals (Bonnet et al., 1981).

2.6.2 Overview of Calculating Reliability. To be usefully clinically, a measurement tool must be valid and reliable (Walmsley & Amell, 1996). A reliable measure ensures that any changes observed are due to the experimental treatment and not error variability, attributable to multiple trials or multiple days (Carson & Kroll, 1970). Error associated with a measurement tool can significantly affect analysis and interpretation of results obtained using this tool. The assessment of this error is therefore crucial and can be accomplished by calculating an index of reliability (Shrout & Fleiss, 1979). Reliability is the consistency or repeatability of a measurement and is expressed as a ratio of the variance in true scores/variance in obtained scores. The variance in obtained scores can be partitioned into a true component and an error component (Portney & Watkins, 1993; Carlson & Kroll, 1970). Thus, reliability is equal to:

$$R = \frac{\sigma_T^{2}}{\sigma_T^{2} + \sigma_E^{2}}$$

Where σ_T^2 is the estimate of variance in true scores, and σ_E^2 is the error variance estimate.

This conceptualization is theoretical, as the true and error components of a data set can never truly be known. However, in essence, if the true variance in a measure is large, the error term will account for a smaller portion of the total observed variance. Thus, a measure is considered reliable if a greater proportion of the total variance is accounted for by the true score (Portney & Watkins, 1993). There are several formulas for calculating a reliability index (Carlson & Kroll, 1970). The primary difference between these formulas is the method of partitioning total variance into true and error components (Feldt & McKee, 1958).

2.6.3 Calculation Using Direct Correlations. Equations providing a correlation coefficient are traditionally used as an indication of reliability (Walmsley & Amell, 1996). Such methods include the calculation of the Pearson product-moment coefficient, or the Spearman rank coefficient. These methods involve calculating the correlation between a measurement on one day and the same measurement on another day. These methods assume that factors such as fatigue, mental state and motivation are constant within an individual on one day, but are variable from day to day (Feldt & McKee, 1958). Each of these factors is assigned to the error term, limiting the correlation to the extent that these factors follow this trend (Feldt & McKee, 1958). Components of variation, therefore, are not efficiently partitioned into true and error (Portney & Watkins, 1993). The values obtained through this type of analysis reflect a covariance as they compare deviations from the means of two measurements (Portney & Watkins, 1993). These methods are therefore limited by the fact that they are sensitive to the range of values obtained with the measure, but are insensitive to systematic differences in the means (Walmsley & Amell, 1996). In other words, if subjects who score high on day 1 also score high on day 2, and subjects who score low on day 1 also score low on day 2, a strong product-moment correlation will exist between the measurements on day 1 and day 2. However, the magnitude of change in the scores from day 1 to day 2 is not considered. It is therefore possible to demonstrate a high correlation even if the repeatability of the scores is low (Portney & Watkins, 1993). Further analysis using a t-test would need to be done to determine if the scores from one trial to the next are significantly different. Additionally, this method is limited to comparing one factor over two trials and is therefore not the most effective method of calculating reliability. The methods of calculating reliability described above do not efficiently partition components of variation into true and error (Portney & Watkins, 1993). They also limit analysis to two data sets at one time. These methods, therefore, do not represent the most effective methods of assessing reliability.

2.6.4 Benefits of the Intraclass Correlation Coefficient. The Intraclass Correlation Coefficient (ICC) provides a more comprehensive method of assessing reliability. The ICC is the reliability of two or more measurements on the same target (Shrout & Fleiss, 1979). It is not restricted by having the same number of raters or restricted to interval or ratio data, as are other reliability measures (Walmsley & Amell, 1996). This is important in clinical and research settings, where the mean of several trials across several days are often used to assess excitability. Reliability as measured by the ICC(2,k) uses mean scores, which are better estimates of the true scores (Portney & Watkins, 1993). It is calculated using the mean square variances obtained from an analysis of variance (Gabriel, 2000; Shrout & Fleiss, 1979; Carlson & Kroll, 1970). In this calculation true score variance is associated with the variance between subjects. As subjects become more consistent at reproducing their own scores, the variance between subjects increases. That is, the differences in variance between subjects become more pronounced, increasing the true score variance and therefore the reliability. The ICC is therefore sensitive to both the order and the magnitude of differences in means (Walmsley & Amell, 1996). It also offers the advantage of partitioning error variance into many components, depending on the experimental design and takes into consideration aspects other than random error, which contribute to variance (Portney & Watkins, 1993). Thus, the general form of the ICC formula is as follows:

$$ICC = \frac{S_T^{\;2}}{S_E^{\;2} + S_F^{\;2}}$$

Where S_T^2 is the estimate of variance in true scores, S_E^2 is the estimate of error variance, and S_F^2 is the estimate of variance in aspects of interest to the measure. This is similar to the general equation for reliability, except the estimate of the true score variance in the denominator of this equation is separated into different facets in the S_F^2 term. Typically the error variance in the ICC is the variability that is associated with multiple trials performed over multiple days (Gabriel, 2000).

2.6.5 Calculation and Interpretation of the Intraclass Correlation Coefficient. While the above-equation represents the general form of the ICC, six different equations for calculating the ICC exist (Portney & Watkins, 1993). Each of these equations falls into one of three models, and is specific to the experimental design (Walmsley & Amell, 1996; Shrout & Fleiss, 1979). The ICC model that is used is determined by the purpose of the investigation. Model 1 is used when each subject is rated by a randomly assigned set of raters from a larger sample of raters. This model is used for generalizability of a particular sample and is rarely used in clinical reliability studies (Portney & Watkins, 1993). Model 2 is used when each subject is rated by the same, randomly assigned raters. This model allows the investigator to make generalizations about the results to a larger population (Portney & Watkins, 1993). Model 3 is applied when the reliability of particular raters is the primary interest of the investigation. In this model raters are not randomly assigned and the results are fixed to that particular sample of raters. This method is often used to assess inter-rater reliability (Portney & Watkins, 1993).

In addition to selecting the appropriate model, the appropriate equation must also be selected. Similar to the models, each of the six equations is specific to experimental design and can be interpreted differently (Walmsley & Amell, 1996; Shrout & Fleiss, 1979). Each form of the equation is denoted by two numbers as follows: ICC (a, k). The first number represents the ANOVA model employed and the second number represents either a single measure (1), or the mean of (k) measurements (Walmsley & Amell, 1996). In choosing the appropriate form of the equation, the following three guidelines are used: (1) is a one-way or two-way ANOVA employed, (2) are differences between individuals relevant to the reliability, and (3) are the ratings single ratings or mean ratings (Shrout & Fleiss, 1979).

The values obtained using the ICC range from 0 to 1.00 (Portney & Watkins, 1993), with values closer to 1.00 indicating that the total observed variance is accounted for by true variance (Walmsley & Amell, 1996). Although no concrete guidelines exist to indicate what is acceptable as reliable (Walmsley & Amell, 1996), in general, anything above R=0.70 is considered reliable and anything below R=0.70 is considered to have moderate to low reliability (Shrout & Fleiss, 1979). The ICC considers several sources of variance. As such, it is a more stringent test of reliability than the other methods discussed.

2.7 Mood

Mood is simply defined as how an individual feels at a given moment in time. It has been suggested that mood has two bipolar dimensions, positive and negative affect (Watson & Tellegen, 1985). Expanding on this original idea, it is suggested that mood is anchored by these positive and negative poles, but that mood is a reflection of the intensity and frequency of these dimensions, making it a multidimensional construct, rather than bipolar (Diener et al., 1985). Mood is constantly under the influence of environmental stimuli and can fluctuate over time (Silva & Weinberg, 1982). It is suggested, however, that certain inherent characteristics influence personal affect, making individual mood responses to a particular stimulus unique (McFatter, 1994). In exercise science, the effect of exercise on mood is a widely studied area (for a review, see Yeung, 1996). However, the inverse relationship, the effects of one's mood on performance of an exercise bout has not received the same degree of attention.

Mood can change readily, and increased psychological arousal is thought to increase physiological output (Wilken et al., 2000). Although the exact mechanisms are not clearly defined, it is suggested that these fluctuations are caused by, among other contributions, changes in neurotransmitter release and small changes in cardiac output (Berger et al., 1993). Furthermore, changes in mood may activate brain regions with corticospinal connections. For example, research on mental imagery has shown that merely thinking about movement can alter spinal excitability (Hashimoto & Rothwell, 1999; Bonnet et al., 1997; Gandevia et al., 1997). Although it has not yet been investigated, it is possible that mood can affect H-reflex excitability through similar corticospinal action.

2.7.1 Subjective Exercise Experience Scale (SEES). A stimulus such as an exercise bout, or a session of H-reflex testing will produce a subjective response within participants. In order to understand the emotional responses and their relation to the stimuli, the subjective responses must be measured. Traditionally, the most widely used instruments are the Profile of Mood States (POMS) (McNair et al., 1981) and the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970, as cited in Yeung, 1996). However, these instruments are based on a two-dimensional assessment of state, positive and negative, rather than on a multidimensional assessment. These instruments, therefore, tend to focus on negative affect and skew the responses toward negative states (McAuley & Courneya, 1994). To capture the multidimensional subjective responses to an exercise bout, McAuley and Courneya (1994) developed the Subjective Exercise Experience Scale (SEES). This is a short 12-item self-report questionnaire with subjects responding to questions on a 7-point Likert scale ranging from “not at all” (1) to “very much so” (7). It addresses three subscales of personal affect, Psychological Well-Being (PWB), Fatigue, and Psychological Distress (PD).

In creating this measure, McAuley and Courneya (1994) drew items that reflected subjective feelings associated with exercise from several widely used measures of mood. These several hundred items were then narrowed to 46 items that a group of experts believed would be positively or negatively affected by exercise. This list was then further reduced to 12 items after administering the test to undergraduate students and performing an exploratory factor analysis. They continued by demonstrating convergent and discriminant validity for the measure and showed that the three subscales, PWB, Fatigue, and PD had high reliabilities of α=.86, α =.88 and α =.85, respectively. The SEES, therefore, is a valid, reliable measure of responses to exercise stimuli and its subscales support a multidimensional response anchored by positive and negative poles (McAuley & Courneya, 1994).

2.7 Summary

The monosynaptic reflex is a useful and effective method of investigating spinal pathways and excitability of the reflex arc. For this reason, it is important to develop a reliable, non-invasive technique of using the H-reflex to assess neuromuscular excitability. The traditional methods of using the H-reflex to assess neuromuscular excitability, the maximum amplitude of the H-reflex response, and the amplitude of the response at certain percentages of the maximum amplitude of the M-wave, are limited in accuracy by the collision effect. Funase et al., (1994) have proposed that calculating the slope of the H-reflex stimulus-response curve provides a measure of reflex arc excitability that attempts to avoid this limitation. However, this method is not without its own limitations and it is proposed in this thesis that calculating the peak of the first derivative of the stimulus-response curve may be an even better measure of excitability. Literature supporting the reliability of the traditional methods is scant, while literature supporting the reliability of the two newer methods is non-existent. It is therefore important to investigate the reliability of these measures to gain a better understanding of their measurement properties. To fully understand the measurement properties it is important to investigate intrinsic factors, which may affect these measurements. The idea of mood affecting recordings of the H-reflex is novel and important. A determination of the reliability of these measures and an understanding of factors affecting the measures will help to develop a method of measuring excitability of the H-reflex arc, which can be applied with confidence clinically.

Chapter 3: Materials and Methods

3.1 Participants

Twenty-four participants (12 males and 12 females) free of neurological disorders were studied without remuneration for their time. All procedures used in this investigation have been reviewed and approved by the Brock University Human Ethics Board. All participants were given an orientation to the laboratory and equipment to be used and signed an informed consent document before testing began.

3.2 Measurement schedule and procedures

Each subject completed five days of testing with 24 to 72 hours between each session. The first three test sessions were used to establish baseline. The sensitivity of the measures under consideration were evaluated on the fourth test day when a 60 Hz, 1-mm displacement vibration (G1MRI, General Electric, Stamford, CT) was applied to the Achilles tendon. The fifth test day was then used to re-establish baseline. All testing was done on the right leg with subjects resting prone on a gurney with both feet hanging slightly off the end. During the first session the age, height, weight, and leg length were recorded. The length of the leg was measured from the stimulation site to the most proximal area of the medial malleolus (Braddom & Johnson, 1974). A skin fold measurement was also taken at the recording site, over the soleus.

Diagrams of the experimental set-up are shown in Figures 7 and 8. Prior to electrode placement, the skin on the lower leg was shaved, lightly abraded with NuPrep® and cleaned with alcohol to reduce signal impedance. A bipolar recording electrode (DE-2.1, DelSys Inc., Boston, MA) with an inter-electrode distance of 1 cm was placed over the belly of the soleus, one third of the distance from the gastrocnemius musculotendinous junction to the Achilles tendon (Delagi & Perotto, 1980). Electrical resistance between the two electrodes was maintained below 10 kΩ.

A self-adhesive ground electrode was secured over the medial femoral condyle. The recording electrode contains a pre-amplifier with a common-mode rejection ratio of >80 dB, which amplifies the signal with a fixed gain of 10. The bioamplifier (Bagnoli 4, DelSys Inc., Boston, MA) increased the signal another 1000-times before it was band-passed filtered between 20 and 450 Hz, sent to a 16 bit A/D converter (DI410, DATAQ Instruments, Akron, OH), and sampled at 3 kHz on a Pentium III PC (Seanix Technology Inc., Blaine, WA). The Computer-Based Oscillograph and Data Acquisition System (CODAS, DATAQ Instruments Inc., Akron, OH) was used to collect and store the signal on hard-disk for further analysis.

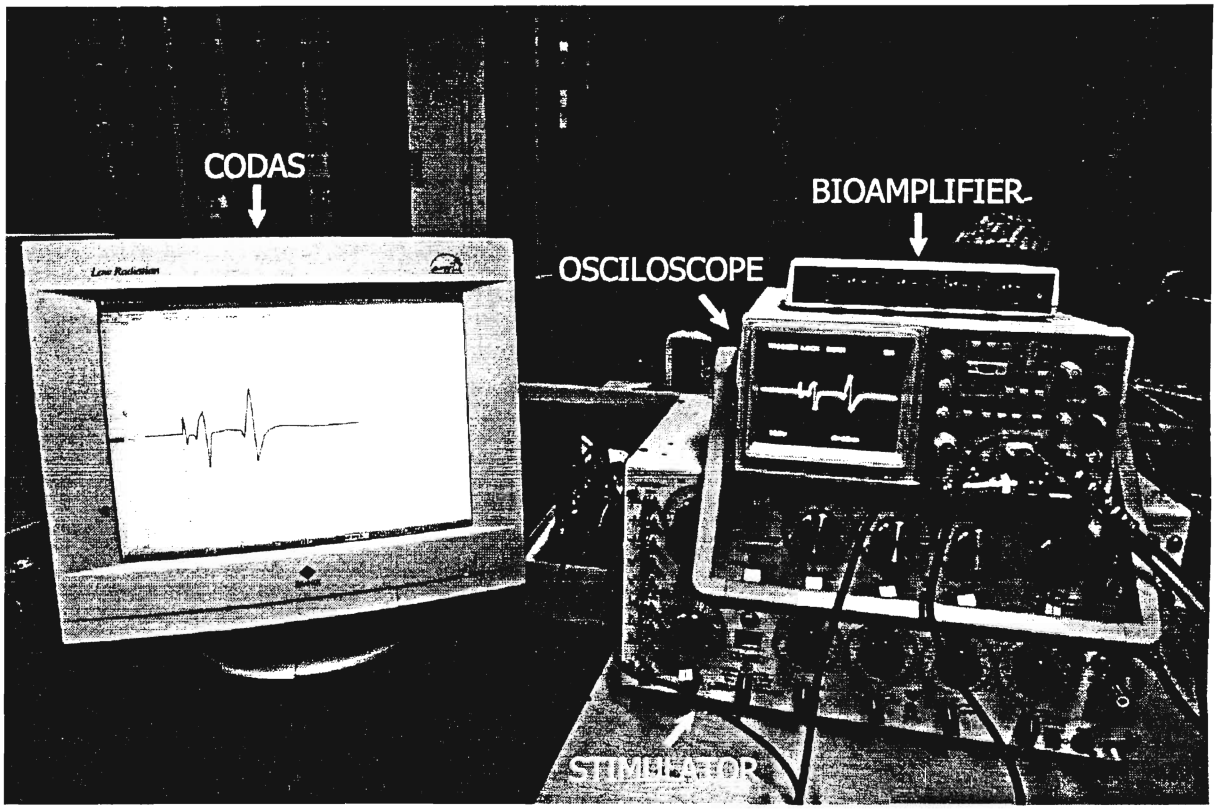

Figure 7. Experimental set-up of equipment.

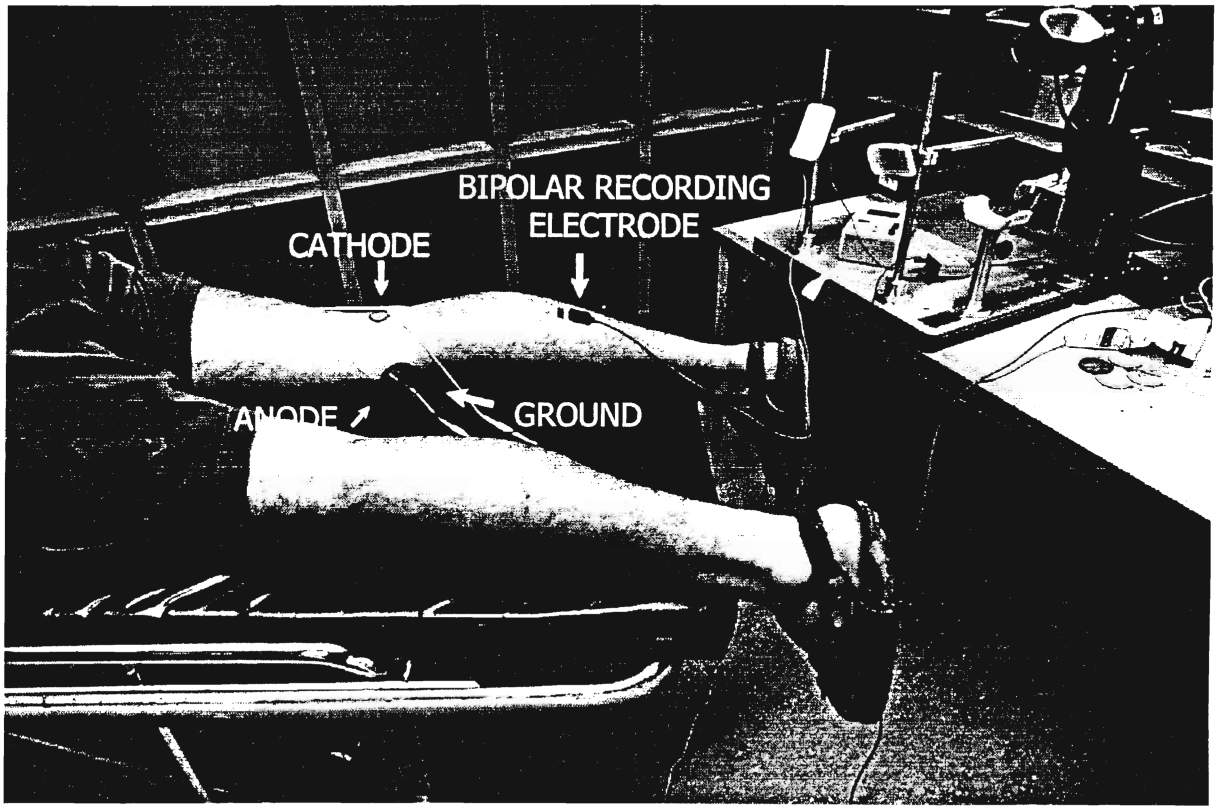

Figure 8. Experimental set-up of subjects.

The H-reflex was evoked with a self-adhesive cathode (Pals Plus, 3.0 cm, Axelgaard, Fallbrook, CA) secured to the skin surface, just above the mid-popliteal crease, over the tibial nerve. A self-adhesive anode (Pals Plus, 5.0 cm, Axelgaard, Fallbrooke, CA) was secured just above the patella. The stimulating electrodes were connected in series with a stimulator (Grass Telefactor S88, Astro-Med, Inc., West Warwick, RI) to deliver a square-wave pulse, 1 ms in duration (Pierrot-Deseilligny & Mazevet, 2000), and then with an isolation unit (Grass Telefactor SIU8, Astro-Med, Inc., West Warwick, RI). At the end of each session the placement of all electrodes was marked with indelible ink, and participants were asked to maintain these marks throughout the duration of the study to ensure consistent electrode placement.

3.3 Stimulation Protocol

Participants were asked to lay comfortably with the head tilted to the right, eyes closed and to remain still throughout the duration of the test session. With the subjects in this position, the tibial nerve was stimulated at low intensities to determine the threshold for the H-reflex. For recordings, the stimulation intensity began below threshold and was gradually increased in 4 V increments until the maximal M response was reached. Then, additional stimulation steps were included to ensure that the maximum M-wave had been achieved and was stable. Five stimuli were delivered at each intensity, with 15 seconds between each stimulus (Williams et al., 1992). This protocol was followed for each of the five days of testing. On test session four, a 60 Hz vibration was applied to the Achilles tendon for the two-second interval immediately preceding tibial nerve stimulation.

3.4 Data Reduction

The mean peak-to-peak amplitude of five trials at each stimulus intensity was calculated (Williams et al., 1992) to construct stimulus-response curves for both the H-reflex and M-wave for each of the five days of testing. Figures 9-13 show the mean H- and M-responses from a representative subject during the sub-threshold to supra-maximal stimulation protocol on each of the five test sessions. It is interesting to note at this point the profound inhibition following vibration on test day 4 (see Figure 12).

The peak-to-peak amplitude of the H-reflex at a stimulus intensity corresponding to 5% of the maximum amplitude of the M-wave (M_{max}) was calculated. First, the stimulus intensity required to generate an M-response that was 5% of the maximum was determined. The amplitude of the H-reflex at that stimulus intensity was then obtained (Hwang, 2002). The method of calculating the slope of the regression line of the stimulus-response curves, advocated by Funase, was reproduced in the following way. The starting point for the regression line was at 5% of the maximum H-reflex amplitude. The end point of the regression line was at the H-reflex amplitude associated with an M-response that was at 10% of the maximum M-wave. This was done to avoid the initial foot of the curve and to avoid any significant collision effects. The slope of the H-reflex stimulus-response curve (H_{slope}) was then calculated from the least squares fit to these data points. Similarly, a least squares regression line was also fit to the data points of the M-wave stimulus-response curve from 10 to 80% of M_{max} and the slope of that line was calculated (M_{slope}). These methods are shown in Figures 13 and 14. Notice that the regression line on the H-reflex curve ends at the same amplitude where the regression line on the M-wave curve begins (10% M_{max}). Both H-reflex and M-wave stimulus-response curves were resampled

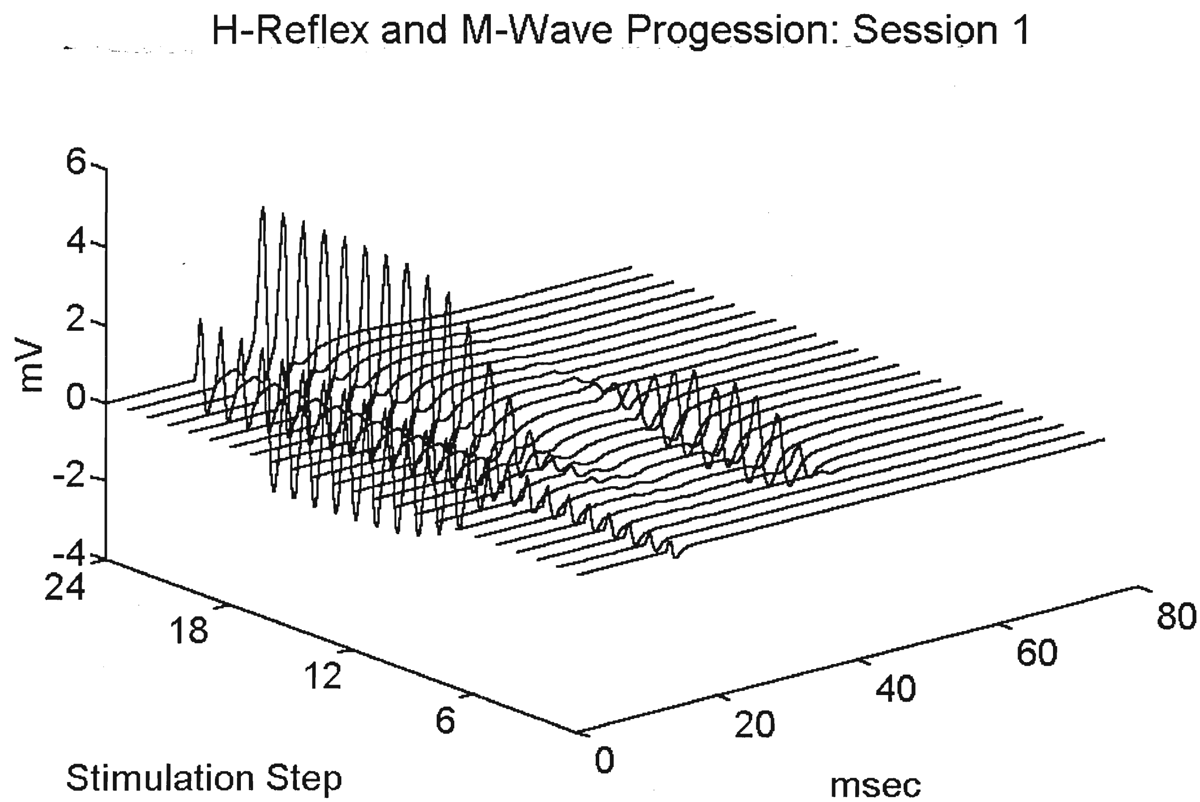

Figure 9. Mean responses of one subject at all stimulus intensities on test day 1. The first waveform, in time, is the stimulus artifact, the second is the M-wave and the third is the H-reflex. The x-axis is time (ms), the y-axis is the amplitude of the response (mV) and the z-axis is the stimulation step, where each step represents an increase in stimulus intensity by 4V.

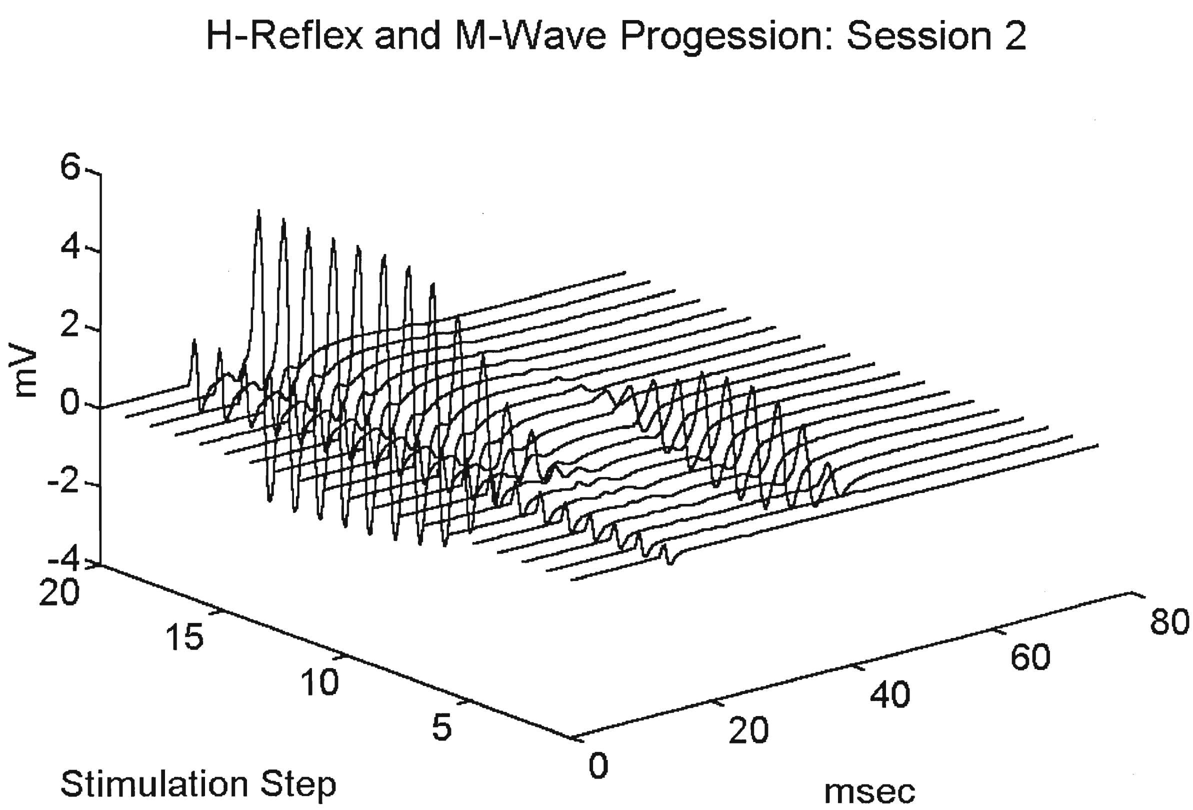

Figure 10. Mean responses of one subject at all stimulus intensities on test day 2. The first wavefor in time, is the stimulus artifact, the second is the M-wave and the third is the H-reflex. The x-axis is time (ms), the y-axis is the amplitude of the response (mV) and the z-axis is the stimulation step, wh each step represents an increase in stimulus intensity by 4V.

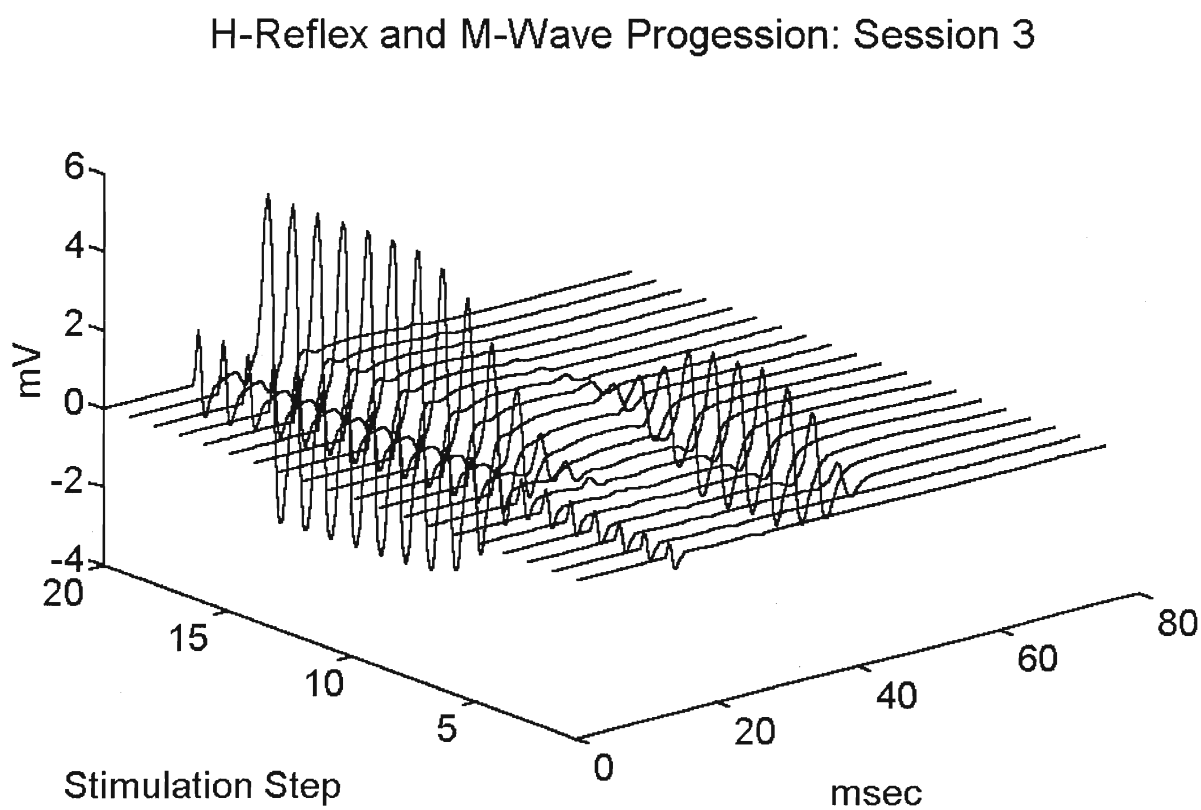

Figure 11. Mean responses of one subject at all stimulus intensities on test day 3. The first wavefor in time, is the stimulus artifact, the second is the M-wave and the third is the H-reflex. The x-axis is time (ms), the y-axis is the amplitude of the response (mV) and the z-axis is the stimulation step, wh each step represents an increase in stimulus intensity by 4V.

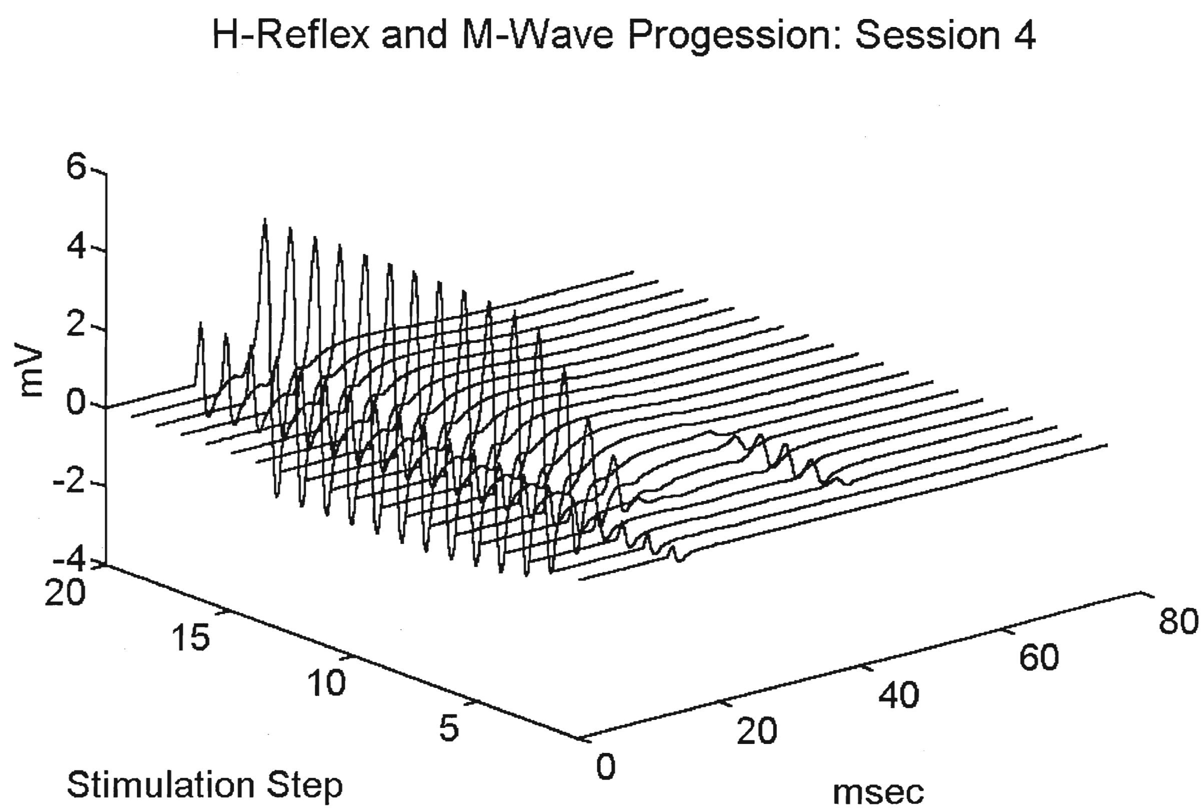

Figure 12. Mean responses of one subject at all stimulus intensities on test day 4. The first wavefor in time, is the stimulus artifact, the second is the M-wave and the third is the H-reflex. The x-axis is time (ms), the y-axis is the amplitude of the response (mV) and the z-axis is the stimulation step, wh each step represents an increase in stimulus intensity by 4V.

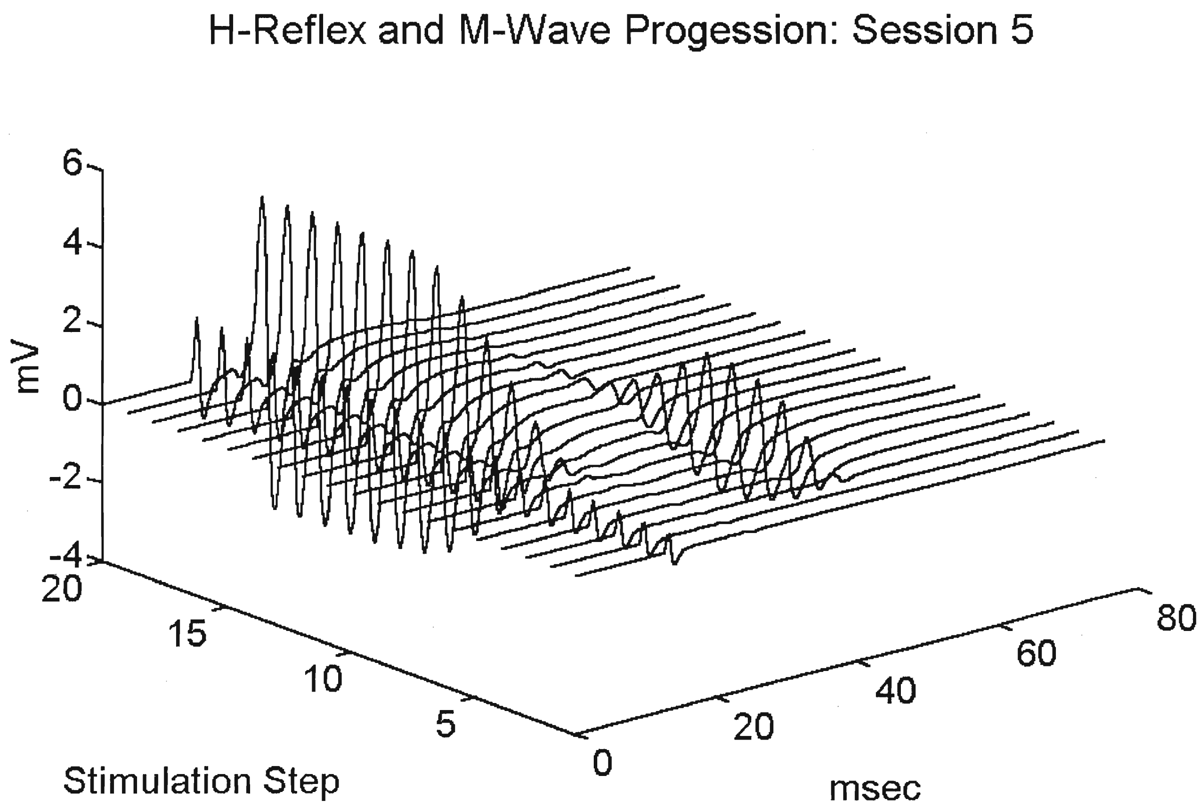

Figure 13. Mean responses of one subject at all stimulus intensities on test day 5. The first wavefor in time, is the stimulus artifact, the second is the M-wave and the third is the H-reflex. The x-axis is time (ms), the y-axis is the amplitude of the response (mV) and the z-axis is the stimulation step, wh each step represents an increase in stimulus intensity by 4V.

up to 100 points using a quadratic interpolating polynomial (Gabriel et al., 2002). They were then numerically differentiated using the finite differences technique to obtain the first derivative. Using this technique the peak of the first derivative of the H-reflex stimulus-response curve (dH/dV_{max}) and the peak of the first derivative of the M-wave stimulus-response curve (dM/dV_{max}) were determined. The peak of the first derivatives of these curves can be observed in Figures 14 and 15.

3.5 Mood

The mood of 20 of the participants (9 males and 11 females) was assessed each day, prior to the stimulation protocol, using the Subjective Exercise Experience Scale (SEES), established by McAuley and Courneya (1994). This is a 12-item, self-report questionnaire aimed at assessing three sub-scales of mood: Psychological well-being (PWB), Fatigue and Psychological Distress (PD). Subjects were asked to respond to the 12 questions on a 7-point Likert scale ranging from "not at all" (1) to "very much so" (7).

3.6 Statistical Analysis

To assess the reliability of the different measures of excitability of the H-reflex arc, the following procedures were used. A one-way repeated measures analysis of variance (ANOVA) was used to test for significant differences in the criterion measures across test days. Tukey's post-hoc test was then used to further evaluate any significant F-ratios (Kirk, 1968). Reliability of the criterion measures was assessed using the intraclass correlation coefficient (ICC). Since each measure represents the mean of five trials, Shrout and Fleiss (1979) suggest the ICC should be calculated according to ICC model (2,k), using the following equation:

$$ICC(2,k) = \frac{BMS - RMS}{BMS + \dfrac{(RMS - EMS)}{n}},$$

where n is the number of subjects, *BMS* is the between subjects mean squares, *RMS* is the residual mean square, and *EMS* is the error mean square obtain from the analysis of variance (ANOVA) table (Portney & Watkins, 2000). Haggard (1958) showed that differences between ICCs could be evaluated by means of the relation between ICC and Fisher's z-statistic:

$$z = \frac{1}{2}\log_e \frac{1+(k-1)\,ICC}{1-ICC},$$

where k is the number of test sessions. The z-statistic allows for the construction of a 95% confidence interval for the observed ICC to determine if significant differences exist at the 0.05 probability level.

To assess the differences between the three H-reflex measures in the sensitivity to vibration on test day 4, the percent change in magnitude between test days 3 and 4 was determined for each participant using the following formula: [(Day3 – Day4) / Day 3] x 100. These data were then subjected to a one-way repeated measures analysis of variance (ANOVA).

Further analysis was done to investigate the possibility of mood as a covariate in assessments of H-reflex excitability. For this purpose, a repeated measures ANOVA with orthogonal polynomials was used for statistical trend analysis of the three subscales of the SEES questionnaire: Psychological Well-being (PWB), Fatigue (F), and Psychological Distress (PD), and each of the measures of the H-reflex: $H_{5\%}$, H_{slope} and dH/dV, across test days. A linear regression analysis was also performed on these data to investigate the possibility of a correlation between the H-reflex measures and any of the subscales of the SEES that showed a significant trend.

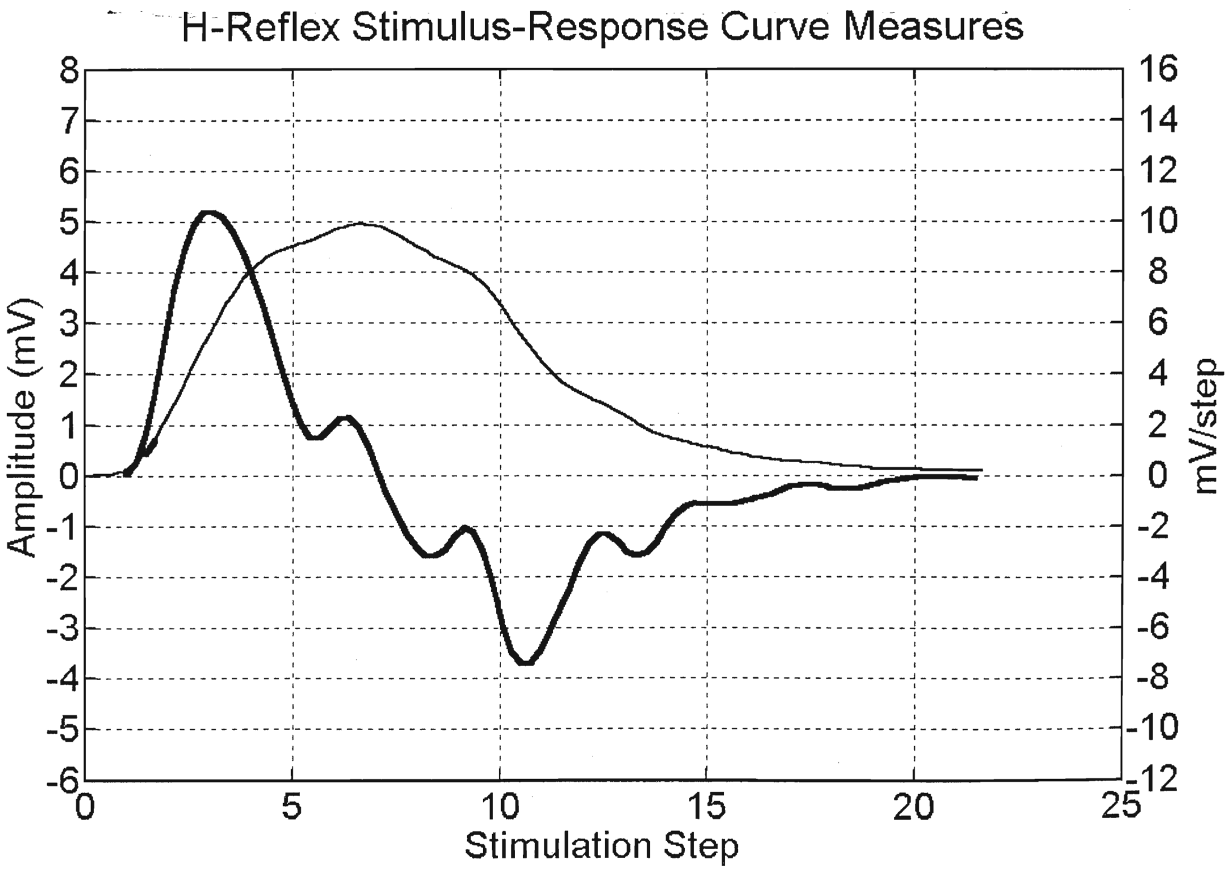

Figure 14. Representative stimulus-response curves from one subject for the H-reflex without vibration. The stimulus-response curve is the thin line, corresponding to the left y-axis. The thick line, corresponding to the right y-axis, shows the first derivative of the curve. The additional straight line shows the regression line of the curve. The x-axis is the stimulation step, where each step represents an increase in stimulus intensity by 4V, the left y-axis is the amplitude of the response (mV) and the right y-axis is the units of the first derivative of the curve (mV/step).

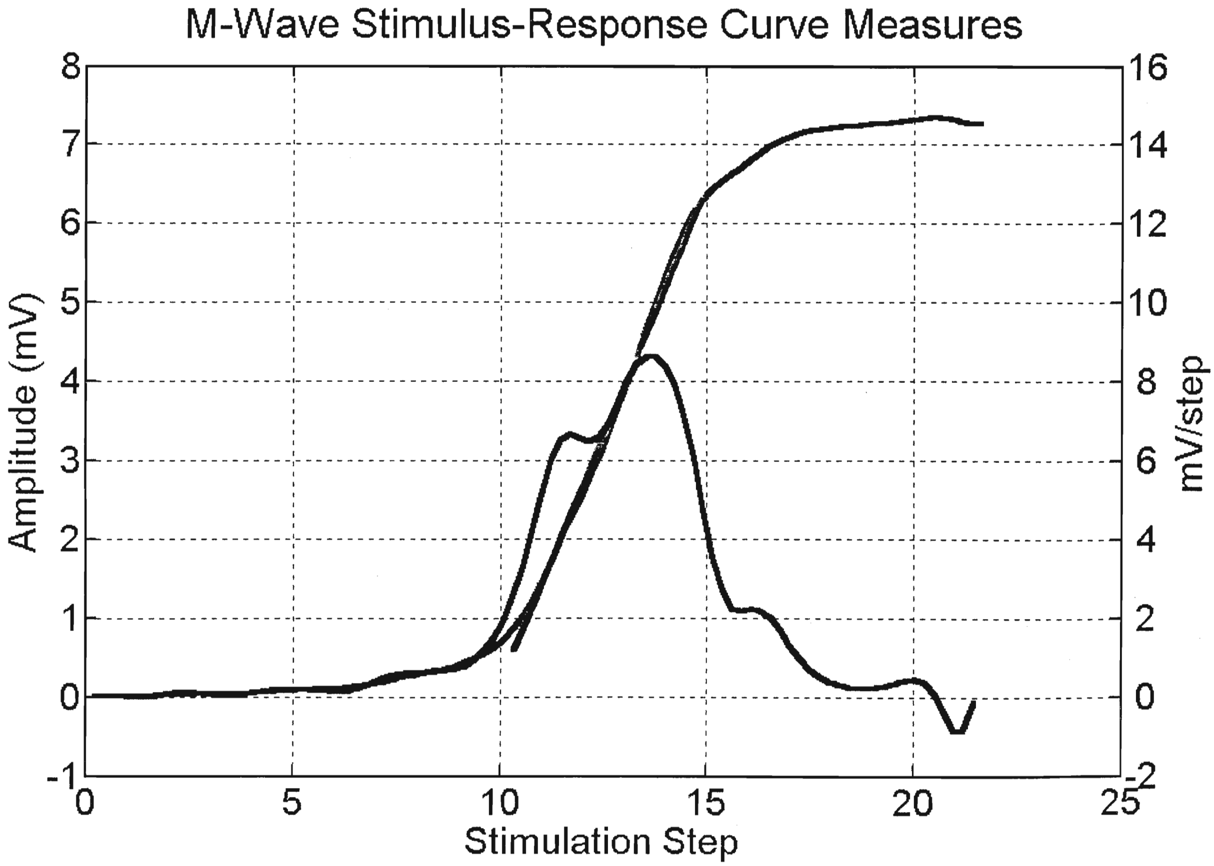

Figure 15. Representative stimulus-response curves from one subject for the M-wave. The stimulus-response curve is the thin line, corresponding to the left y-axis. The thick line, corresponding to the right y-axis, shows the first derivative of the curve. The additional straight line shows the regression line of the curve. The x-axis is the stimulation step, where each step represents an increase in stimulus intensity by 4V, the left y-axis is the amplitude of the response (mV) and the right y-axis is the units of the first derivative of the curve (mV/step).

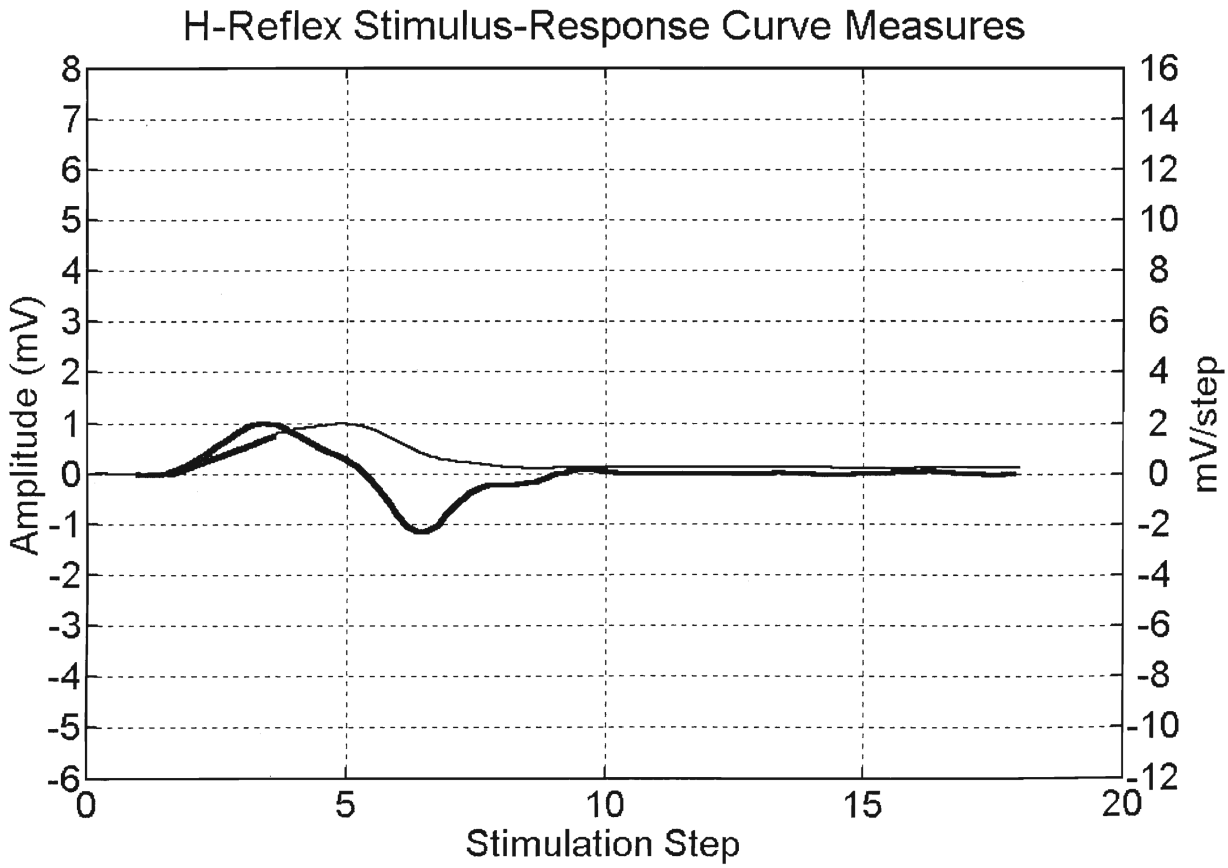

Figure 16. Representative stimulus-response curves from one subject for the H-reflex with vibration. The stimulus-response curve is the thin line, corresponding to the left y-axis. The thick line, corresponding to the right y-axis, shows the first derivative of the curve. The additional straight line shows the regression line of the curve. The x-axis is the stimulation step, where each step represents an increase in stimulus intensity by 4V, the left y-axis is the amplitude of the response (mV) and the right y-axis is the units of the first derivative of the curve (mV/step).

Chapter 4: Results

4.1 Subject Characteristics

Twenty-four participants (12 males and 12 females) completed the five days of testing. Descriptive statistics of relevant physical characteristics are presented in Table 1.

Table 1 – Means (M) and standard deviations (SD) for the physical characteristics of the participants.

Physical characteristics	M±SD
Age (years)	24.17±4.03
Height (cm)	173.7±8.85
Weight (kg)	72.60±17.09
Body Mass Index ($kg{\cdot}m^{-2}$)	25.18±3.54
Skin fold (mm)	10.16±4.75
Leg length (cm)	41.91±3.01

4.2 M-wave measures

Tables 2 and 3 show sample ANOVA tables for M_{max}, outlining the model used for all measures of the H-reflex and M-wave for between groups (male and female) and within subjects comparisons, respectively. Preliminary analysis revealed a significant difference between males and females ($P<0.05$) for the maximum amplitude of the M-wave, but not for Mslope or dM/dV (P's>0.05). The magnitude of the SEMG signal is partly dependent on the size of the muscle. It is therefore not surprising the maximum M-wave amplitude was greater for males than for females (Kent-Braun et al., 2002; Ditor & Hicks, 2000). However, these differences were strictly in magnitude. The means for both groups followed the same pattern across days as reinforced by the non-significant (P's>0.05) day-by-group interaction term. Consequently, the data from males and females were collapsed for further analysis.

Table 2 – ANOVA model used for all measures of the H-reflex and M-wave for between group (male and female) comparisons, showing data from M_{max}.

Source	SS	DF	MS	F	P
Group	58.050	1	58.050	6.131	0.021
Error	208.308	22	9.469		

Table 3 - ANOVA model used for all measures of the H-reflex and M-wave for within subjects comparisons, showing data from M_{max}.

Source	SS	DF	MS	F	P
Day	3.603	4	0.901	3.286	0.015
Day * Group	0.878	4	0.219	0.800	0.528
Error	24.123	88	0.274		

Table 4 presents the means and standard deviations for the measures under consideration. The maximum M-wave amplitude remained virtually unchanged across the five test sessions (P's>0.05) attesting to careful methodological controls. The slope of the M-wave stimulus-response curve exhibited similar stability. The means of the peak of the first derivative of the M-wave stimulus response-curve had slightly more variability but still remained unchanged across the five test sessions, with no significant differences in means across days (P's>0.05).

Table 4 shows the intraclass correlation coefficients (ICC) for M-wave measures. As might be expected, the ICC for M_{max} was excellent at 0.96. The ICC for the slope of the M-wave stimulus-response curve was equally high (0.95). The peak of the first derivative of the M-wave stimulus-response curve had an ICC that was slightly lower though still very good (0.83). The 95% confidence intervals did not reveal any significant differences in the reliability of the measures of the M-wave.

4.3 H-reflex measures

There were no significant gender differences in any measure of the H-reflex and no group by day interaction effects were observed (P's>0.05). The data from males and females were therefore collapsed for further analysis. The means and standard deviations for the H-reflex measures are presented in Table 5.

Significant F-ratios were observed for all H-reflex measures (P's<0.01). The Tukey's post-hoc analysis indicated a slight but non-significant (P's>0.05) decrease in all H-reflex measures from test day one to test day three. The means on test day four however were significantly lower than the means on all other test days (P's<0.01) and returned to baseline on day 5. Otherwise, the means for the H-reflex measures were shown to be stable.

The intraclass correlation coefficients (ICC) for all H-reflex measures are presented in Table 5. Because the vibration was an intentional manipulation of the system, the means from day 4 will inherently lower the reliability of each of the measures. For this reason the intraclass correlation coefficient (ICC) of all measures was calculated with the means from day 4 excluded. The maximum amplitude of the H-reflex had an ICC of 0.79, while the H-reflex amplitude at a stimulus intensity corresponding to 5% of M_{max} had an ICC of 0.85. The slope of the H-reflex stimulus-response curve had an ICC of 0.79. The peak of the first derivative of the H-reflex stimulus-response curve had a higher reliability than the other H-reflex measures with an ICC of 0.89. The 95% confidence intervals (see Table 5), however, did not reveal any significant differences between the ICCs.

Table 4 – Means (M) and standard deviations (SD) for the maximum amplitude of the M-wave (M_{max}); the slope of the M-wave SR curve (M_{slope}); and the peak of the first derivative of the M-wave SR curve (dM/dV). The ICC and its 95% confidence interval (CI) of estimation were calculated for each measure for test days 1,2,3, and 5.

Measures	**Day** 1 *M±SD*	2 *M±SD*	3 *M±SD*	4 *M±SD*	5 *M±SD*	**ICC(2,k)**	**95% C.I.**
M_{max} (mV)	5.8±1.5	5.7±1.5	5.3±1.5	5.6±1.6	5.5±1.8	0.96	$C(0.96 \leq ICC \leq 0.98)$
M_{slope} (units)	0.8±0.5	0.7±0.4	0.7±0.4	0.8±0.5	0.8±0.6	0.95	$C(0.88 \leq ICC \leq 0.97)$
dM/dV (mV/V)	9.4±4.3	8.4±4.1	7.3±2.8	7.4±3.3	8.4±4.8	0.83	$C(0.65 \leq ICC \leq 0.92)$

Table 5 – Means (M) and standard deviations (SD) for the H-reflex amplitude at a stimulus intensity corresponding to 5% of maximum M-wave ($H_{5\%}$); the slope of the H-reflex stimulus-response (SR) curve (H_{slope}); and the peak of the first derivative of the H-reflex SR curve (dH/dV). The ICC and its 95% confidence interval (CI) of estimation were calculated for each measure for test days 1,2,3, and 5.

Measures	**Day**						
	1	2	3	4	5		
	M±SD	*M±SD*	*M±SD*	*M±SD*	*M±SD*	**ICC(2,k)**	**95% C.I.**
$H_{5\%}$ (mV)	2.9±0.9	2.6±1.1	2.5±1.0	0.8±0.7	2.6±1.4	0.84	$C(0.68 \leq \text{ICC} \leq 0.93)$
H_{slope} (units)	1.1±0.5	0.9±0.4	0.9±0.5	0.2±0.2	0.8±0.5	0.79	$C(0.59 \leq \text{ICC} \leq 0.90)$
dH/dV (mV/V)	11.2±6.0	10.0±4.0	9.0±4.3	3.3±3.7	7.2±6.3	0.89	$C(0.76 \leq \text{ICC} \leq 0.95)$

4.4 Vibration

All measures of the H-reflex decreased on day 4 when vibration was applied to the Achilles tendon, indicating similar sensitivities to this perturbation. The largest decrease was observed in the H-reflex amplitude at a stimulus intensity associated with 5% of M_{max}. However, there was no significant difference (P>0.05) between the four H-reflex measures in the percent change between test days 3 and 4. The group responses (mean ± standard deviation) are presented in Table 6. The magnitude of the decrease in the H-reflex stimulus-response curve for a representative subject is illustrated in Figures 13 and 15.

Table 6 – Means (M) and standard deviations (SD) percent decrease from Day 3 to Day 4 in each of the H-reflex measures.

Measure	% Decrease M±SD
H_{max}	64±23
$H_{5\%}$	70±24
H_{slope}	69±43
dH/dV	59±34

4.5 Mood

All three measures of the H-reflex yielded similar results in the trend and regression analysis. Therefore, only the data from the best-established measure, $H_{5\%}$, are reported. Mean scores for $H_{5\%}$ and the three subscales of the SEES are presented in Table 7. There was a significant cubic trend for $H_{5\%}$ (P<0.01). H-reflex amplitude decreased from 3.0 ± 0.9 mV on session one to 2.5 ±1.0 mV on session three. A profound reduction was observed on session four (0.8 ± 0.7 mV) when vibration was applied to the Achilles tendon. H-reflex amplitude then returned to baseline on session five (2.6 ± 1.4 mV). The SEES sub-scales for psychological well-being and fatigue remained unchanged throughout the five testing days. There was however a significant cubic trend for psychological distress ($P<0.05$). Psychological distress increased from 1.55 ± 0.65 on session one to 1.84 ± 0.95 on session three. Session four showed a return to baseline (1.56 ± 0.67), and then another marked increase on session five (2.1 ± 1.3). Regression analysis revealed a correlation between $H_{5\%}$ and psychological distress of $r=0.394$ was not significant ($P>0.05$).

Table 7 - Mean scores of $H_{5\%}$, Psychological well-being (PWB), Fatigue and Psychological Distress (PD) on the five test days.

	Mean Score				
Measure	Day 1	Day 2	Day 3	Day4	Day 5
$H_{5\%}$	2.96	2.66	2.46	0.79	2.60
PWB	5.59	5.62	5.22	5.45	5.10
Fatigue	3.38	3.02	3.42	2.92	3.26
PD	1.55	1.85	1.84	1.56	2.08

Chapter 5: Discussion

5.1 M-Wave Measures

The M-response is a direct motor response. As such, it is not affected by physiological changes at the level of the spinal cord. It is typically measured in H-reflex studies to ensure stability in the test conditions. The amplitude of the maximum M-response varies among individuals and can range from 4-8 mV (Schieppati, 1987). The M_{max} values obtained in this investigation are in line with these expected values, with the group mean peak-to-peak values ranging from 5.3 to 5.8mV across days. M_{max} is believed to represent the recruitment of all motor units within the muscle. The amplitude of this response therefore, should not change if test conditions are consistent. If there are no observed changes in the M-response, but changes are observed in the H-reflex, we can be confident that the changes in the H-reflex are due to differences in excitability of reflex arc, not changes in the experimental conditions. While the absence of changes in M_{max} is an ideal condition, changes in this measure have been observed. Crone et al. (1999) have reported decreases in M_{max} as large as 35.6% in healthy subjects. However, these decreases are typically observed when the methodology involves evoking maximal M-responses several times over the course of one test session. In the current investigation M_{max} was evoked a limited number of times with little variation in amplitude. In fact, the mean M_{max} values had a very small range within subjects across days and an extremely high reliability. Previous studies have reported inter-session reliability of M_{max} to range from 0.87 to 0.97 (Palmieri et al., 2002; Handcock et al., 2001; Williams et al., 1992). The results of the current investigation are in line with these data, with ICC values for the three measures ranging from 0.83 to 0.96. It is interesting to note that M_{slope} had an equally high reliability while dM/dV was slightly lower. This difference suggests that dM/dV is slightly more sensitive to small differences in the peak rate of change in the M-wave stimulus-response curve than is M_{slope}.

5.2 H-Reflex Measures

5.2.1 Comparative Amplitude Values. Calculating the peak of the first derivative of the stimulus-response curve is a novel method of estimating H-reflex excitability. Comparisons of the values obtained in the current investigation with previous findings, therefore, cannot be made. The maximum peak-to-peak amplitude and the slope of the regression line of the stimulus-response curve, however, can be compared with previous findings. On average, the maximum peak-to-peak amplitude of the H-reflex is approximately 50% that of the maximum peak-to-peak amplitude of the M-response, but has been found to range from 24-100% (Zehr, 2002; Schieppati, 1987). The results of the current investigation follow this trend. With the exception of day four (vibration) the maximum peak-to-peak amplitudes of the H-reflexes were 30-100% M_{max}. The slope values are also in line with values previously reported by Funase et al. (1994). While Funase et al. (1994) do not present actual numbers, the figure of mean slope values depicts that the H_{slope} and M_{slope} were both around 1.0 at rest, and that H_{slope} was greater than M_{slope}. Our data are very similar to this, with the mean H_{slope} ranging from 0.8 to 1.1 across the five days (except test day 4) and M_{slope} ranging from 0.7 to 0.8. Thus, we were able to successfully replicate the methods of Funase et al. (1994).

5.2.2 Comparative Reliability Values. Literature reporting the reliability of H-reflex measures is limited. In general, the few investigations that exist, report that H-reflex recordings are very robust. It has been shown that H-reflex recordings from the soleus are very reliable within a subject across trials, with Pearson *r*-values greater than 0.95 (Handcock et al., 2001, Williams et al., 1992). Furthermore, the maximum peak-to-peak amplitude within individuals across test days has also been found to be reliable with ICC(3,1) values as high as 0.99 reported for the triceps surae (Handcock et al., 2001, Morelli et al., 1990). While the ICC values obtained in the current investigation were not quite as high as previously reported, the measures were found to be reliable. Discrepant findings may result from the different measurements investigated and the statistical procedures chosen. Most of the previous investigators report Pearson r values, while we chose the ICC(2,k).

While the Pearson r is a widely used method for calculating reliability, it is limited in its effectiveness. It is a comparison of deviations from a mean. As such it is sensitive to the range of the means, but not to systematic differences between the means (Walmsley & Amell, 1996). It is also limited to comparing only two measurements at a time (Portney & Watkins, 1993). The statistical procedures employed in this investigation, the calculation of the ICC, allowed for a better estimate of reliability. The ICC allows for comparison of more than two mean scores at a time (Portney & Watkins, 1993). Additionally, the ICC is sensitive to both the range of means and to systematic differences between them (Carlson & Kroll, 1970). Although Morelli et al. (1990) also used the ICC, a different form of the equation was employed in the present investigation. Morelli et al. (1990) calculated the ICC using the equation ICC(3,1), indicating that reliability was estimated based on a three-way ANOVA of a single measure. In the present investigation a one-way repeated measures ANOVA was performed and the mean of 5 trials was used in the calculation of reliability with the equation ICC(2,k). The use of mean scores across trials provides a better estimate of the true score (Portney & Watkins, 1993) making the statistical methods of this investigation a more stringent test of reliability.

5.2.3 Possible Sources of Variation. Small differences in the resting activation level of motoneurons have been observed (Funase & Miles, 1999), which can cause variation in the amplitude of the H-reflex across trials. This variation can affect the reliability across days. However, this was controlled for in the present investigation by averaging five trials at each stimulus intensity. Such averaging has been shown to produce reliable H-reflexes between 0.864 and 0.998 (Handcock et al., 2001, Hopkins et al., 2000, Herbert & Boucher, 1998 Williams et al., 1992).

Other possible factors that can affect the reliability of H-reflex recordings, in general, include inter-stimulus interval, subject position and electrode placement. Large variations in H-reflex amplitude can be observed if the inter-stimulus interval is not sufficient for relaxation of the motoneurons. In other words, enough time must be allowed between stimuli for the sensory and motor neuron activity levels to return to baseline. If the time between stimuli is very short (10 ms) the response can be inhibited (Zehr, 2002). If it is longer than 10 ms, but shorter than 10 s, the response can be facilitated (Zehr, 2002). A sufficient interval of 15 seconds, however, was used in this investigation (Pierrot-Deseilligny & Mazevet, 2000). All subjects in this investigation were lying prone. In this position, the H-reflex can be reliably recorded (Hopkins et al., 2000). However, the position of the head, knee, and ankle of the subjects can also affect H-reflex recordings (Al-Jawayed et al., 1999). These positions, therefore, were standardized for each subject and subjects were instructed to remain motionless throughout each session. The recorded responses, and thus the reliability, could also be altered by even slight changes in the position of the recording and/or stimulating electrodes. To avoid this, the placement of all electrodes was carefully marked at the end of each session. This particular factor would have less impact on the proposed method of estimating excitability than on the traditional methods.

Because the first derivative of the curve reflects a rate of change, rather than an absolute amplitude of the response, it is less likely to be affected by slight changes in electrode placement. Keeping all of these variables constant provides confidence that the observed changes from day to day in all measures were physiological, rather than experimental, in origin.

5.2.4 Inhibition. The H-reflex is often referred to as a monosynaptic reflex, suggesting that the Ia sensory fibres make single synaptic connections with the motor fibres in the ventral horn of the spinal cord. The amplitude of the H-reflex therefore represents the ability of the sensory fibres to depolarize the α-motoneurons, or the excitability at the level of the motoneuron pool. Several researchers, however, suggest that the H-reflex does not quantify motoneuron excitability as it is influenced by presynaptic and recurrent inhibition (Kernell & Hultborn, 1990; Morita et al., 1998; Zehr, 2002). The activity in two inhibitory pathways is believed to be responsible for this inhibition. The first is autogenic presynaptic inhibition. Activation of the sensory fibres can also cause activation of group Ia and Ib afferents, which activate group I inhibitory interneurons (Pierrot-Deseilligny & Mazevet, 2000; Lin et al., 2002; Marchand-Pauvert et al., 2002). This will cause presynaptic inhibition of the α-motoneurons, effectively decreasing the amplitude of the H-reflex. The second is recurrent inhibition produced by the activation of Renshaw cells. Renshaw cells can be recurrently activated through activation of motoneurons and these cells can selectively inhibit α-motoneurons (Pierrot-Deseilligny & Mazevet, 2000; Earles et al., 2002; Marchand-Pauvert et al., 2002).

While the contribution of these pathways to inhibition of the H-reflex is generally accepted, there is no direct evidence supporting their roles (Marchand-Pauvert et al., 2002). It has been demonstrated that not all motoneurons have recurrent collaterals and not all recurrent collaterals activate Renshaw cells (Earles et al., 2002). Additionally, the excitation involved in the H-reflex is exclusively due to the effects of Ia sensory fibres on α-motoneurons (Lin et al., 2002). Analysis of the latency of discharge of motoneurons involved in the H-reflex indicates that the first motoneurons recruited, near threshold of the M-wave, are recruited through monosynaptic connections (Magladery et al., 1951). Consistent with this is the suggestion that the level of inhibition increases with increasing stimulus intensity (Earles et al., 2002). Therefore, at low stimulus intensity the contribution of inhibitory pathways is small, making the activation of the α-motoneurons truly monosynaptic. The observed responses at low intensity stimulations, therefore, may truly reflect excitability of the motoneuron pool. The purpose of the method of assessing the H-reflex proposed by Funase et al. (1994) was to avoid the effects of collision. Perhaps this method provides an additional advantage in that it avoids not only collision, but also inhibition. The method proposed in this investigation, calculating the peak of the first derivative of the stimulus-response curve would share this advantage. For all subjects in the current investigation the peak of the first derivative of the stimulus-response curve occurred at or before the threshold of the M-wave. This suggests that this method also avoids both collision and inhibition. Thus, the rate of change methods of assessing the H-reflex provide a better representation of excitability of the reflex arc than more traditional methods, such as H_{max}.

5.3 Vibration

It is suggested that muscle spindle afferent fibres respond to vibratory stimulation of the agonistic tendon between 1 and 100 Hz (Calvin-Figuiere et al., 1999). Such vibration has been found to have inhibitory effects on the H-reflex (Herbert & Boucher, 1998; Hilgevoord et al., 1996; Zehr, 2002). It has been reported that vibration of the Achille's tendon can decrease the amplitude of the H-reflex by 20-75% (Hilgevoord et al., 1996). Vibratory inhibition of the H-reflex observed in the present investigation falls within this range. The mean percent decreases across all subjects in this investigation ranged from 59%, for the peak of the first derivative of the curve, to 70%, for the maximum amplitude of the H-reflex. All measures then returned to baseline on day five, suggesting that these inhibitory effects are not long-term.

It has been demonstrated that the de-recruitment of motor units with vibration follows the same order as the recruitment pattern (Desment & Godaux, 1978). According to Henneman's size principle, motor units are recruited in an orderly fashion from smallest to largest (Henneman, 1957). Recordings from single motor units have indicated that tendon vibration causes inhibition of the smallest motor units first, at low stimulus intensities, and inhibits motor units of gradually increasing size with gradually increasing stimulus intensity (Desment & Godaux, 1978). Although the exact mechanism of this depression is not understood, it is generally accepted that vibration of an antagonist muscle tendon causes an increase in presynaptic inhibition of the Ia motoneurons supplying the agonist muscle (Zehr, 2002, Calvin-Figuiere et al., 1999).

5.4 Comparison with Traditional Measures

Although the 95% confidence interval revealed no significant differences in the reliability of the measures, the first derivative of the stimulus-response curve had a higher ICC than the slope of the regression line, the maximum amplitude, or the amplitude at 5% M_{max}. Although the differences were not significant, it has been demonstrated that the peak of the first derivative is comparable to the other measures. This proposed method of measuring excitability of the H-reflex not only has a higher ICC, but also offers additional advantages over the other methods.

The maximum peak-to-peak amplitude of the H-reflex is often used as a method of estimating the excitability of the reflex arc. However, it is often desirable to evoke H-reflexes at a constant level to maintain control between groups (Earles et al., 2002). The theory supporting this method is that stimulation of a consistent number of motoneurons is associated with stimulation of a consistent number of sensory fibres (Capaday, 1997). To accomplish this, the maximum amplitude of the M-wave is determined and the stimulus intensity is then set to a level that evokes a certain percentage of that maximum (Hwang, 2002). The peak-to-peak amplitude of the H-reflex at that intensity (in the present investigation this was 5%) is then calculated. However, these traditional measures are limited by the collision effect (Funase et al., 1994). When H_{max} is observed the threshold for the M-wave has been reached. This direct activation of the motor fibres produces antidromic potentials, which collide with the orthodromic sensory signal, preventing some of that sensory signal from reaching the muscle. The actual level of activity within the spinal cord, therefore, is not truly represented at the muscle. This collision is less significant when evoking the H-reflex at a stimulus intensity corresponding to 5% M_{max} (Basmajian & DeLuca, 1985). This may explain the lower ICC value of H_{max} as compared to $H_{5\%}$.

A possible explanation for the peak of the first derivative having a higher ICC value than both H_{max} and $H_{5\%}$ is that it provides a rate of change, rather than an amplitude. This makes it a more robust measure, with less variability between trials. Because it is less likely to be affected by even slight changes in experimental conditions, for example, small differences in electrode placement, it is more likely to yield more consistent values, making it more reliable. The slope of the regression line, as proposed by Funase et al. (1994) also provides a rate of change, but the peak of the first derivative offers a more parsimonious fit for a sigmoid-shaped curve than a least-squares linear fit. Additionally, the methods employed by Funase et al. (1994) to fit the regression line and calculate the slope, were somewhat ambiguous. Funase et al. (1994) state that the regression line was fit to points on the H-reflex stimulus-response curve that were below the threshold for the M-response. The number of stimulation steps separating the thresholds of the H-reflex and the M-response, however was variable within subjects across trials. To allow for more consistency in the number of points across trials, we analyzed the H-reflex stimulus-response curve from 5% of H_{max} up until 10% of M_{max}. This algorithm allowed for a similar number of data points across subjects and trials for the least-squares linear fit and minimized the effects of collision. Since the H_{slope} means observed in this study were nearly identical to those reported by Funase et al. (1994), the algorithm was successful.

5.5 Mood

Mood is a relatively long-lived subjective feeling (Ketai, 1975). It is composed of all emotions, physical states, cognitive states, and the external environment (Mayer et al., 1994). Despite its relative stability it has been shown to be influenced by environmental stimuli (Ketai, 1975; Rusting, 1998; Russell, 2003). It has also been suggested that changes in mood have a closely-linked physiological counterpart (Russell, 2003). Such alterations in mood have been linked with such physiological changes as changes in heart rate and neurotransmitter, and hormone levels (Berger et al., 1993). While it has been demonstrated that changes in both mood and H-reflex amplitude are correlated with changes in exercise performance (Raglin et al., 1995), a direct link between mood and the H-reflex amplitude has not been made.

It has been suggested that anxiety may negatively affect the neuromuscular system during force production (Behm & Button, 2002). Although this is an intriguing suggestion, the construct of anxiety, nor any other psychological construct, has been evaluated in such an investigation. Because the idea of psychological factors affecting neuromuscular responses have never been empirically measured, a general measure of mood, the Subjective Exercise

Experience Scale (SEES), with a subscale measuring psychological distress was chosen in this investigation. The psychological distress subscale has been shown to be positively correlated with both the negative states on PANAS and with measures of state anxiety (McAuley & Courneya, 1994). While the subscales of fatigue and psychological well-being remained stable across the five test days, psychological distress showed a significant cubic trend in its day-to-day changes. The amplitude of the H-reflex at 5% M_{max} also showed a significant cubic trend across the five days.

Both psychological distress and $H_{5\%}$ showed a profound decrease on day 4 when vibration was applied to the Achilles tendon. Such a decrease was expected in the H-reflex with vibration, due to increases in presynaptic inhibition, as previously discussed. The reason for the decrease in distress, however, is somewhat more elusive. The subjects were aware of the perturbation and had been oriented to the sensation prior to filling out the SEES on day 4. It is therefore possible that the introduction of a relaxing stimulus, as opposed to the electrical stimulation of the nerve, decreased any distressful feelings the subjects had about the stimulation protocol. Both psychological distress and $H_{5\%}$ then returned to baseline on day 5, when this perturbation was removed. The trend analysis indicated a significant decrease in $H_{5\%}$ from day 1 to day 3. Ideally, the amplitude of the H-reflex should remain constant across test days when there is no perturbation of the system. This decrease in the amplitude of the H-reflex was associated with an increase in psychological distress from day 1 to day 3. Possible reasons for this increase in distress include subjects becoming bored with the protocol, or subjects becoming more anxious about the electrical stimulation. Although the mechanisms for the observed changes in $H_{5\%}$ and psychological distress are not known, it is possible that they are related.

This is very preliminary data investigating trends in the means and does not show a direct correlation between any of the subscales of mood and the amplitude of the H-reflex. However, the similarity in the trends of $H_{5\%}$ and psychological distress suggests that the negative valence of mood, as measured by the SEES may be related to changes in H-reflex amplitude. This provides evidence that measures of mood, or perhaps measures of state anxiety, may be useful in H-reflex investigations. Further research should be done using measures of state anxiety and larger subject pools to validate mood or anxiety as a covariate.

Chapter 6: Summary and Conclusions

6.1 Summary and Significance

Assessment of excitability using H-reflex recordings is a beneficial clinical and research tool. It is important, therefore, to understand the characteristics of its measurement. We need to be confident that observed alterations in the H-reflex are associated with physiological mechanisms and not random variation in its measurement. It has been demonstrated here that calculating the peak of the first derivative of the stimulus-response curve has comparable reliability and sensitivity to more traditional methods of assessment. This new method further offers two distinct advantages over traditional measures. It avoids the limitation of collision, as suggested by Funase et al. (1994) and possibly avoids the limitation of inhibitory inputs. It is also less affected by slight changes in experimental conditions, as it reflects a rate of change rather than an absolute amplitude, making it a more robust measure of excitability. The method of calculating slope of the regression line of the stimulus-response curve, as proposed by Funase et al. (1994) also offers these advantages. However, their method is limited in its poor approximation of the beginning portion of the H-reflex stimulus-response curve, which has a sigmoid shape.

Although further research needs to be done to fully understand the effects of mood on the H-reflex, some preliminary suggestions are offered here. It was demonstrated that the mean scores of a negative pole of mood, psychological distress, varied significantly across days. The trends in the changes of the mean followed a trend similar to that of changes in the amplitude of the H-reflex at 5% M_{max}. Although there was no significant correlation between the two measures, both had a significant cubic trend. The inexplicable changes in both psychological distress and $H_{5\%}$ from day 1 to day 3 suggests that the changes in the two measures may be related. It is therefore possible that measures of anxiety would be useful in studies of the H-reflex. However, further investigation involving a larger number of subjects must be conducted before any firm conclusions can be drawn about this possible relationship.

6.2 Conclusions

The peak of the first derivative of the H-reflex stimulus-response curve was as sensitive as other measures to changes in the neuromuscular system produced by vibration. Although the first derivative of the stimulus-response curve had a higher ICC value than the slope of the regression line of the curve and the amplitude measures, its reliability was statistically similar. This newly proposed method, however, does provide other advantages over the traditional methods. It was additionally determined that a sub-scale of mood assessment, psychological distress, may be a useful covariate when assessing H-reflex recordings. Although psychological well-being and fatigue remained stable, changes in psychological distress followed a pattern similar to changes in the amplitude of H-reflex recordings across days.

Chapter 7: References

Al-Jawayed IA, Sabbahi M, Etnyre BR, and Hasson S. The H-reflex modulation in lying and semi-reclining (sitting) position. *Clin Neurophysiol* 1999; 110: 2044-2048.

Aymard C, Katz R, Lafitte C, Lo E, Penicaud A, Pradat-Diehl P, and Raoul S. Presynaptic inhibition and homosynaptic depression: A comparison between lower and upper limbs in normal human subjects and patients with hemiplegia. *Brain* 2000; 123: 1688-1702.

Basmajian JV, and DeLuca CJ. *Muscles Alive: Their Function Revealed by Electromyography* (5th ed.). Baltimore, MD: Williams & Wilkins, 1985.

Behm DG, and Button DC. The effect of stimulus anticipation on the interpolated twitch technique. *Proc of the Can Soc Exerci Physiol*, Canada, 2002; 27: S4.

Berger BG, Owen DR, and Man F. A brief review of literature and examination of acute mood benefits of exercise in Czechoslovakian and United States swimmers. *Int J Sport Psych* 1993; 24: 130-150.

Bonnet M, Decety J, Jeannerod M, and Requin J. Mental simulation of an action modulates the excitability of spinal pathways in man. *Cog Brain Res* 1997; 5: 221-228.

Bonnet M, Requin J, and Semjen A. Human reflexology and motor preparations. *Ex Sport Sci Rev* 1981; 9: 119-157.

Braddom RI, and Johnson EW. Standardization of H-reflex and diagnostic use in S1 radiculopathy. *Arch Phys Med Rehabil* 1974; 55: 161-166.

Calvin-Figuiere S, Romaiguere P, Gilhodes JC, and Roll JP. Antagonist motor responses correlate with kinesthetic illusions induced by tendon vibration. *Exp Brain Res* 1999; 124: 342-350.

Capaday C. Neurophysiological methods for studies of the motor system in freely moving human subjects. *J Neurosci Methods* 1997; 74: 201-218.

Carson BR, and Kroll W. The use of anlaysis of variance in estimating reliability of isometric elbow flexion strength. *Res Quart* 1970; 29(3): 279-293.

Chaffin DB, Andersson GBJ, and Martin B. *Occupational Biomechanics* (3rd ed.). New York, NY: Wiley & Sons, 1999.

Crone C, Johnsen LL, Hultborn H, and Orsnes G.B. Amplitude of the maximum motor response (M_{max}) in human muscles typically decreases during the course of the experiment. *Exp Brain Res* 1999; 124: 265-270.

Delagi EF, and Perotto A. *Anatomic guide for the electromyographer*, (2nd ed.). Springfield: Thomas, 1980.

Desmedt JE, and Godaux E. Mechanism of the vibration paradox: Excitatory and inhibitory effects of tendon vibration on single soleus muscle motor units in man. *J Physiol* 1978; 285: 197-207.

Diener E, Larsen RJ, Levine S, and Emmons RA. Intensity and frequency: Dimensions underlying positive affect. *J Personality Soc Psych* 1985; 48: 1253-1265.

Ditor DS, and Hicks AL. The effect of age and gender on the relative fatigability of the human adductor pollicis brevis muscle. *Can J Physiol Pharmacol* 2000; 78: 781-790.

Earles DR, Dierking JT, Robertson CT, and Koceja DM. Pre- and post-synaptic control of motoneuron excitability in athletes. *MSSE* 2002; 34(11): 1766-1772.

Feldt LS, and McKee ME. Estimation of the reliability of skill tests. *Res Quart* 1958; 29(3): 279-293.

Funase K, Imanaka K, and Nishihira Y. Excitability of the soleus motoneuron pool revealed by the developmental slope of the H-reflex as reflex gain. *Eletromyogr Clin Neurophysiol* 1994; 34: 477-489.

Funase K, and Miles TS. Observations on the variability of the H-reflex in human soleus. *Muscle Nerve* 1999; 22: 341-346.

Gabriel DA, Proctor D, Engle D, Nair S, Vittone J, and An K-N. Application of the LaGrange polynomial in skeletal muscle fatigue analysis. *Res Quart Ex Sport* 2002; 73: 168-174.

Gabriel DA, Basford JR, and An K-N. Training-related changes in the maximal rate of torque development and EMG activity. *J Electrmyogr Kinesiol* 2001; 11: 123-129.

Gabriel DA, Basford JR, and An K-N. Neural adaptations to fatigue: implications for muscle strength and training. *Med Sci Sport Ex* 2000; 33(8): 1354-1360.

Gabriel DA. Reliability of SEMG spike parameters during concentric contractions. *Electromyogr Clin Neurophysiol* 2000; 40: 423-430.

Gandevia SC, Wilson LR, Inglis JT, and Burke D. Mental rehersal of motor tasks recruits alpha-motoneurones but fails to recruit human fusimotor neurons selectively. *J Physiol* 1997; 505.1: 259-266.

Haggard EA. Intraclass correlation and the analysis of variance. New York, NY: Dryden Press Inc, 1958.

Handcock PJ, Williams LR, and Sullivan SJ. The reliability of H-reflex recordings in standing subjects. *Electromyogr Clin Neurophysiol* 2001; 41(1): 9-15.

Hashimoto R, and Rothwell JC. Dynamic changes in corticospinal excitability during motor imagery. *Exp Brain Res* 1999; 125: 75-81.

Hwang IS. Assessment of soleus motoneuron excitability using the joint-angle dependent H-reflex in humans. *Neurophysiol* 1996; 13(5): 366-384.

Henneman E. Relation between size of neuron and their susceptibility to discharge. *Science* 1957; 126: 1345-1346.

Herbert J, and Boucher JP. Effect of manual segmental vibration on neuromuscular excitability. *J Man Physiol Ther* 1998; 21(8): 528-533.

Higashi T, Funase K, Kusano K, Tabira T, Harada N, Sakakibara A, and Yoshimura T. Motoneuron pool excitability of hemiplegic patients: Assessing recovery stages by using H-Reflex and M-Response. *Arch Phys Med Rehabil* 2001; 82: 1604-1610.

Hilgevoord AAJ, Koelman JHTM, Bour LJ, Ongerboer and de Visser BW. The relationship between the soleus H-reflex amplitude and vibratory inhibition in controls and spastic subjects. I. Experimental results. *J Electromyogr Kinesiol* 1996; 6: 253-258.

Hopkins JT, Ingersoll CD, Cordova ML, and Edwards JE. Intrasession and intersession reliability of the soleus H-reflex in supine and standing positions. *Electromyogr Clin Neurophysiol* 2000; 40(2): 89-94.

Hugon M. Polysynaptic and monsynaptic reflexes evoked in the short head of the femoral biceps in normal man. *Electroencephalogr Clin Neurophysiol* 1970 28(1): 89.

Hultborn H, Meunier S, Pierrot-Deseilligny E, and Shindo M. Changes in presynaptic inhibition of Ia fibres at the onset of voluntary contraction in man. *J Physiol (London)* 1987; 389: 757-772.

Hwang IS. Assessment of soleus motoneuron excitability using the joint-angle dependent H-reflex in humans. *J Electromyogr Kinesiol* 2002; 12: 351-366.

Kagamihara Y, Hayashi A, Okuma Y, Nagaoka M, Nakajima Y, and Tanaka R. Reassessment of H-reflex recovery curve using the double stimulation procedure. *Muscle Nerve* 1998; 21: 352-360.

Kamen G, and Caldwell GE. Physiology and interpretation of the electromyogram. *J Clin Neurophysiol* 1996; 13(5): 366-384.

Kent-Braun JA, Ng AV, Doyle JW, and Towse TF. Human skeletal muscle responses vary with age and gender suring fatigue due to incremental isometric exercise. *J Appl Physiol* 2002; 93: 1813-1823.

Kernell D, and Hultborn H. Synaptic effects on recruitment gain: a mechanism of importance for the input-output relations of motoneuron pools? *Brain Res* 1990; 507: 176-179.

Ketai, R. Affect, mood, emotion, and feeling: Semantic considerations. *Am J Psychiatry* 1975; 132(11): 1215-1217.

Kirk RE. Experimental design: Procedures for the Behavioral sciences, Belmont: Brooks/Cole Publishing Company, Chaps 4-5, 99-150, 1968.

Kohn AF, Floeter MK, and Hallett M. Presynaptic inhibition compared with homosynaptic depression as an explanation for soleus H-reflex depression in humans. *Exp Brain Res* 1997; 116: 375-380.

Magladery JW, Porter WE, Park AM, and Teasdall RD. Electrophysiological studies on nerve and reflex activity in normal man IV. Two-neurone reflex and identification of certain action potentials from spinal roots and cord. *Bull Johns Hopkins Hospital* 1951; 88:499-519.

Mayer JD, Salovey P, Gomberg-Kaufman S, and Blainey K. A broader conception of mood experience. *J Personality Soc Psych* 1991; 60(1): 100-111.

McAuley E, and Courneya KS. The Subjective Exercise Experience Scale (SEES): Development and preliminary validation. *J Sport Ex Psych* 1994; 16: 163-177.

McFatter RM. Interactions in predicting mood from extraversion and neroticism. *J Personality Soc Psyc* 1994; 66: 570-578.

McNair PM, Lorr M, and Droppleman LF. *POMS Manual* (2nd ed.). San Diego: Education and Industrial Testing Service, 1981.

Miller TA, Newall AR, and Jackson DA. H-reflexes in the upper extremity and the effects of voluntary contraction. *Electromyogr Clin Neurophysiol* 1995; 35: 121-128.

Minium EW. Statistical reasoning in psychology and education (2nd ed.). New York, NY: John Wiley & Sons, 1978.

Morelli M, Sullivan SJ, and Seaborne DE. Comparrison of human triceps surae H-reflexes obtained from mid and distal recording sites. *Electromyogr Clin Neurophysiol* 1990; 30(3): 181-186.

Morin C, Pierrot-Deseilligny E, and Hultborn H. Evidence for presynaptic inhibtion of muscle spindle afferents in man. *Neurosci Lett* 1984; 44: 137-142.

Morita H, Petersen N, Christensen LOD, Sinkjaer T, and Nielsen J. Sensitivity of H-reflexes and stretch reflexes to presynaptic inhibition in humans. *J Neurophysiol* 1998; 80: 610-620.

Palmieri RM, Hoffman MA, Ingersoll CD. Intersession reliability for H-reflex measurements arising from the soleus, peroneal, and tibialis anterior musculature. *Int J Neurosci* 2002; 112: 841-850.

Pierrot-Deseilligny E, and Mazevet D. The monosynaptic reflex: a tool to investigate motor control in humans: Interest and limit. *Neurophysiol Clin* 2000; 30: 67-80.

Portney LG, Watkins MP. Foundations of clinical research: Applications to practice (2nd Ed.). Upper Saddle River, NJ: Prentice Hall Health, 2000.

Portney LG, and Watkins MP. *Foundations of clinical research: Applications to practice*. Norwalk, CN: Appleton & Lange, 1993.

Preston DC, and Shapiro BE. *Electromyography and neuromuscular disorders: Clinical-electrophysiologic correlations*. Boston, MA: Butterworth-Heineman, 1998.

Raglin JS, Koceja DM, Stager M, and Harms CA. Mood, neuromuscular function, and performance during training in female swimmers. *Med Sci Sports Exerc* 1996; 28(3): 372-377.

Rau G, and Disselhorst-Klung C. Principles of high spatial resolution surface EMG (HSR-EMG): Single motor unit detection and application in the diagnosis of neuromuscular disorders. *J Electromyogr Kinesiol* 1997; 7: 233-239.

Roll JP, Vedel JP, and Ribot E. Alteration of proprioceptive messages induced by tendon vibration with a microneurographic study. *Exp Brain Res* 1989; 76(1): 213-222.

Russell JA. Core affect and the psychological construct of emotion. *Psych Rev* 2003; 110(1): 145-172.

Rusting CL. Personality, mood, and cognitive processing of emotional information: Three conceptual frameworks. *Psych Bul* 1998; 124(2): 165-196.

Schalow G, and Zach GA. Nerve compound action potentials analysed with the simultaneously measured single fibre action potentials in humans. *Electromyogr Clin Neurophysiol* 1994; 34(8): 451-465.

Schieppati M. The Hoffmann reflex: A means of assessing spinal reflex excitability and its descending control in man. *Prog Neurobiol* 1987; 28: 345-376.

Shrout PE, and Fleiss JL. Intraclass correlations: Uses in assessing rater reliability. *Psych Bullet* 1979; 86: 420-428.

Silva JM, and Weinberg RS (Eds). *Psychological foundations of sport*. Champaign, IL: Human Kinetics, 1982.

Soderber G. Selective topics in surface electromyography for use in the occupational setting: Expert perspectives. *DHHS (NIOSH)*, Publication No. 91-100, Washington, DC, NIOSH.

Spielberger CD, Gorsuch RL, and Lushene RE. *State-Trait Anxiety Inventory for Adults (Form X)*. Palo Alto, CA: Consulting Psychologists Press, 1970.

Walmsley RP, and Amell TK. The application and interpretation of intraclass correlations in the assessment of reliability in isokinetic dynamometry. *Isokin Ex Sci* 1996; 6: 117-124.

Watson D, and Tellegen A. Toward a consensual structure of mood. *Psych Bull* 1985; 98(2): 219-235.

Wilken JA, Smith BD, Tola K, and Mann M. Trait anxiety and prior exposure to non-stressful stimuli: effects on psychophysiological arousal and anxiety. *Int J Psychophysiol* 2000; 37: 233-242.

Williams LRT, Sullivan SJ, Seaborne DE, and Morelli M. Reliability of individual differences for H-reflex recordings. *Electromyogr Clin Neurophysiol* 1992; 32: 42-49.

Winter DA. *Biomechanics and motor control of human movement* (2^{nd} ed.). New York, NY: Wiley & Sons, Inc., 1990.

Yaar I. Normative ranges by curve fitting to raw data. *Clin Neurophysiol* 1999; 110: 556-563.

Yeung RR. The acute effects of exercise on mood state. *J Psychom Res* 1996; 40(2): 123-141.

Zehr EP. Considerations for use of the Hoffmann reflex in exercise studies. *Eur J Appl Physiol* 2002; 86: 455-468.

APPENDIX A

Ethics Approval

Brock University

Senate Research Ethics Board **Extensions 3205/4315, Room C315**

FROM: David Butz, Chair
Senate Research Ethics Board (REB)

TO: Dr. David Gabriel, Physical Education
Dr. Joffre Mercier, Biology
Anita Christie, Biology

FILE: **00-034, Christie**

DATE: **October 30, 2000**

The Brock University Research Ethics Board has reviewed the research proposal:

"Realiability of the slope of the Hoffmann reflex stimulus-response curve for the upper extremity."

The Subcommittee finds that your proposal conforms to the Brock University guidelines set out for ethical research.

* *Accepted as is* (Consider changing "principle investigator" to "principal investigator", and "free to withdrawal" to "free to withdraw")

Please note: If Changes or Modifications are required to this approved research, they must be reviewed and approved by the committee. If so, please complete form #5 - ***Request for Ethics Clearance of a Revision or Modification to an Ongoing application for Ethics Review of Research with Human Participants*** and submit it to the Chair of the Research Ethics Board. You can download this form from the Office of Research Services or visit the web site:
http://www.BrockU.CA/researchservices/mainethicsformpage.html

DB/ll

APPENDIX B

Informed Consent Document

BROCK UNIVERSITY DEPARTMENT OF APPLIED HEALTH SCIENCES
Informed Consent Form

Title of Study: Reliability of the slope of the Hoffmann reflex stimulus-response curve for the soleus.

Principal Investigator: Anita Christie
Graduate Student
Applied Health Sciences
Brock University
500 Glenridge Avenue
St. Catharines, Ontario
L2S 3A1
Phone: (905) 688-5550 ext. 3880
E-mail: anitac@niagara.com

Supervising Professor: David A. Gabriel, Ph.D.
Assistant Professor Biomechanics
Department of Physical Education
Brock University
500 Glenridge Avenue
St. Catharines, Ontario
L2S 3A1
Phone: (905) 688-5550 ext. 4362
E-mail: dgabriel@arnie.pec.brocku.ca

This study has been reviewed and approved by the Brock research Ethics Board (File #00-034). The Brock Research Ethics Board requires written informed consent from participants prior to participation in a research study, so that they are aware of the nature of the risks of participation and can decide to participate or not to participate in a free and informed manner. You are asked to read the following material to ensure that you are informed of the nature of this research study and how you will participate in it if you consent to do so. Signing this form will indicate that you have been so informed and that you give consent.

Introduction:

You are being asked to participate in research being conducted by Anita Christie. This research is designed to examine neuromuscular activity in a muscle of the lower leg following stimulation of an associated nerve. The ultimate goal of this study is to assess the measurement properties and the reliability of a proposed method of analyzing neuromuscular excitability.

You will come to the Biomechanics Laboratory in the Physical Education Complex at Brock University for a total of five test sessions over a one-week period. Each test session will last approximately two hours. All testing will be conducted by Anita Christie. During each session you lay flat on your stomach on a testing table, with your head tilted to one side. You will allow the nerve above the back of your knee to be electrically stimulated up to 220 times during any given session. This does not require the insertion of any needles and is safe for you. The electrode for stimulating the nerve will be taped to the skin surface and will deliver very brief electrical impulses through the skin. Changes in muscle electrical activity will be monitored by placing two electrodes over both a muscle in the back of the lower leg and a muscle in the front of your lower leg, with a distance of three centimetres between the two electrodes. This is similar to the electrocardiogram wherein surface electrodes are placed on the chest to monitor electrical activity of the heart muscle.

On the fourth day of testing a vibrator will be held against the Achilles tendon and will be used to massage the tendon for two seconds before nerve stimulation occurs. This procedure is safe for-you and is painless.

Risks and Discomforts:

It is not possible to predict all possible risks or discomforts that a participant may experience in any research study. Based on previous studies with similar methodology, the present investigator anticipates no major risks or discomforts will occur in the present study.

Participants sometimes experience mild discomfort when their skin is gently cleaned and rubbed with a mild abrasive in preparation for electrode placement. On occasion, some subjects may experience skin irritation associated with the placement of the electrodes. This is usually very mild and goes away in a few hours, or a day.

Electrical stimulation to the nerve will be brief and is not usually perceived as painful. On rare occasion, some individuals are unable to tolerate electrical stimulation to the nerve and feel faint; it is impossible to know in advance if you are such an individual. The test will be discontinued at the onset of nausea or fainting. You will already be lying down during the test.

Alternatives:

Needle electromyography is an alternative method for studying muscular activity. Surface electrodes, therefore, represent the least invasive means of recording the electrical activity of the muscle.

Voluntary Participation:

Participation in this study is voluntary. Refusal to participate will not result in any consequences to you. You will inform the investigator, Anita Christie, of your intention to withdraw prior to removing yourself from this study.

Termination of Participation:

Participation in this research study may be terminated under the following circumstances. The investigator, Anita Christie, may discontinue your involvement in the study at any time if it is felt to be in your best interest, if you do not comply with the study requirements, or if the study is stopped. You will be informed of any changes in the nature of the study or in the procedures described, if they occur.

Potential Benefits:

You will receive no direct benefits from participating in this study. However, you should know that your willingness to serve as a subject for this experiment will help a Brock University researcher and other scientists develop new, non-invasive methods of assessing neuromuscular activity.

Costs and Compensation:

The cost of the test and procedures are free. You will not receive any form of compensation for your participation in this study.

Confidentiality:

Although data from this study may be published, confidentiality of information concerning all participants will be maintained. Any personal information related to you will be kept in a locked office, to which only the Faculty Supervisor will have access. Names of participants or materials identifying participants will not be released without written permission, except as such release is required by law.

Persons to Contact with Questions:

The investigator will be available to answer any questions concerning this research, now or in the future. You may contact the investigator, Anita Christie, by telephone at (905) 680-0431 (home) or at (905) 688-5550 ext. 3880 (Biomecahnics Laboratory), or by email at anitac@niagara.com. Alternatively, you may contact the supervising professor, David A. Gabriel, Ph.D., by telephone during office hours at (905) 688-5550 ext. 4362, or by email at dgabriel@arnie.pec.brocku.ca. Also, if questions arise about your rights as a research subject, you may contact the Director of the Office of Research Services at (905) 688-5550 ext. 3205. If you wish to speak with someone not involved in the study, please call Dr. Anna Lathrop, Chair of the Department of Physical Education at (905) 688-5550 ext. 4361.

Consent to Participate:

Certify that you have read all the above, asked questions and received answers concerning areas you did not understand, and have received satisfactory answers to these questions. You willingly give consent for participation in this study. A copy of the consent form will be given to you.

Name of Participant (please print): ______________________________

____

Signature of Participant Date (day/month/year)

In addition to the considerations described in this document, the investigator fully intends to conduct all procedures with the subject's best interest uppermost in mind, to ensure the subject's safety and comfort.

I have fully explained the procedures of this study to the above volunteer. I believe that the person signing this form understands what is involved in this study and voluntarily agrees to participate.

____

Anita Christie Date (day/month/year)

If you wish to receive a summary of the results from this study, please complete the following information. A summary will be sent to you once all data have been analyzed. This summary will be maintained as part of your file that will be stored in a locked office. When and if the data are published, you may also have access to the results on the world wide web. Type the name of the Faculty Supervisor (David Gabriel) as part of a search on Medline at: http://www.nlm.nih.gov/databases/freemedl.html.

Name of Participant: ______________________________

Mailing Address:

____

Street, P.O. Box, Rural Route #, Apt. #

____

City, Province, Postal Code

APPENDIX C

Subjective Exercise Experience Scale (SEES)

Subject #:__________ Day #: _________

Subjective Exercise Experience Scale (SEES)

The SEES is a 12-item adjective scale requiring the subject to rate current feelings along a 7-point intensity scale ranging from "Not at all" to "Very much so". The instrument provides three subscale scores that are sensitive to the stimulus properties of exercise. These include: positive well-being, psychological distress and fatigue. Evidence for internal-consistency, internal validity and factorial validity have been shown.

Directions: Please indicate the degree to which you are experiencing each feeling RIGHT NOW

1. Great

   1 2 3 4 5 6 7
   Not at all Moderately Very much so

2. Awful

   1 2 3 4 5 6 7
   Not at all Moderately Very much so

3. Drained

   1 2 3 4 5 6 7
   Not at all Moderately Very much so

4. Positive

   1 2 3 4 5 6 7
   Not at all Moderately Very much so

5. Crummy

   1 2 3 4 5 6 7
   Not at all Moderately Very much so

6. Exhausted

   1 2 3 4 5 6 7
   Not at all Moderately Very much so

7. Strong

   1 2 3 4 5 6 7

Not at all			Moderately			Very much so

8. Discouraged

1	2	3	4	5	6	7
Not at all			Moderately			Very much so

9. Fatigue

1	2	3	4	5	6	7
Not at all			Moderately			Very much so

10. Terrific

1	2	3	4	5	6	7
Not at all			Moderately			Very much so

11. Miserable

1	2	3	4	5	6	7
Not at all			Moderately			Very much so

12. Tired

1	2	3	4	5	6	7
Not at all			Moderately			Very much so

APPENDIX D

ANOVA Tables for M_{max}

Between Groups (Males and Females)

Source	SS	DF	MS	F	P
Group	58.050	1	58.050	6.131	0.021
Error	208.308	22	9.469		

Within Subjects

Source	SS	DF	MS	F	P
Day	3.603	4	0.901	3.286	0.015
Day * Group	0.878	4	0.219	0.800	0.528
Error	24.123	88	0.274		

APPENDIX E

ANOVA Tables for M_{slope}

Between Groups (Males and Females)

Source	SS	DF	MS	F	P
Group	2.529	1	2.529	3.045	0.095
Error	18.275	22	0.831		

Within Subjects

Source	SS	DF	MS	F	P
Day	0.122	4	0.031	0.940	0.445
Day * Group	0.024	4	0.006	0.188	0.944
Error	2.862	88	0.033		

APPENDIX F

ANOVA Tables for dM/dV

Between Groups (Males and Females)

Source	SS	DF	MS	F	P
Group	92.285	1	92.285	1.850	0.188
Error	1097.466	22	49.885		

Within Subjects

Source	SS	DF	MS	F	P
Day	69.007	4	17.252	2.725	0.034
Day * Group	28.818	4	7.205	1.138	0.344
Error	557.106	88	6.331		

APPENDIX G

ANOVA Tables for H_{max}

Between Groups (Males and Females)

Source	SS	DF	MS	F	P
Group	0.933	1	0.933	0.498	0.488
Error	41.189	22	1.872		

Within Subjects

Source	SS	DF	MS	F	P
Day	80.100	4	20.025	40.308	0.000
Day * Group	0.707	4	0.177	0.356	0.839
Error	43.718	88	0.497		

APPENDIX H

ANOVA Tables for $H_{5\%}$

Between Groups (Males and Females)

Source	SS	DF	MS	F	P
Group	0.772	1	0.772	0.244	0.626
Error	69.610	22	3.164		

Within Subjects

Source	SS	DF	MS	F	P
Day	71.136	4	17.784	29.729	0.000
Day * Group	2.004	4	0.501	0.837	0.505
Error	52.642	88	0.598		

APPENDIX I

ANOVA Tables for H_{slope}

Between Groups (Males and Females)

Source	SS	DF	MS	F	P
Group	0.016	1	0.016	0.032	0.860
Error	11.358	22	0.516		

Within Subjects

Source	SS	DF	MS	F	P
Day	9.415	4	2.354	20.976	0.000
Day * Group	0.317	4	0.079	0.707	0.589
Error	9.874	88	0.112		

APPENDIX J

ANOVA Tables for dH/dV

Between Groups (Males and Females)

Source	SS	DF	MS	F	P
Group	60.162	1	60.162	0.818	0.375
Error	1617.123	22	73.506		

Within Subjects

Source	SS	DF	MS	F	P
Day	924.079	4	231.020	17.773	0.000
Day * Group	60.877	4	15.219	1.171	0.329
Error	1143.832	88	12.998		

APPENDIX K

Raw Data for Psychological Well-Being (PWB)

PWB

Subject #	**Day1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
1	5.5	5.5	5.25	4.75	5.25
2	6.5	5.5	6	6.5	6.5
3	6.5	4.75	7	7	7
4	4.5	4	4.5	5	5
5	6.25	5.75	6	5.5	5.75
6	4.5	5	4.5	4.25	4.5
7	6	7	6.25	5.75	5.5
8	5.25	6.25	2	5	5
9	5	6	6	6	6
10	5	5.25	4.25	4	4.25
11	6	5.5	4	5.25	4.25
12	5.25	5	5.5	4.25	4
13	6.25	6.5	7	6.25	6.25
14	4.25	4.5	5	4.75	4
15	5.75	6.75	4.25	4.75	2.5
16	6.25	6.25	6.5	6.75	6.75
17	5.75	5.5	4.25	6	2.5
18	5.5	6	5.5	6	5.5
19	5.75	6.25	5	5.25	5.75
20	6	5.25	5.75	6	5.75

APPENDIX L

Raw Data for Fatigue

Fatigue

Subject #	Day1	Day 2	Day 3	Day 4	Day 5
1	5	4.25	3.5	3.75	3.75
2	4.5	4.5	4	2	2.75
3	2	4.75	1	3.75	3
4	3.25	5.5	5.5	2.25	2.25
5	4.25	4	2.75	3	4
6	2	2.25	3	2	2
7	1	1	2.25	1.5	1
8	1.75	1	6	1.5	1
9	5	2.5	3	5	4
10	3.25	3.25	3.75	4	4
11	3	2.25	4.5	4	1.75
12	5.25	4	3.5	4.5	4
13	1	1	1	1	1
14	4.5	3.75	3.75	4.5	4.75
15	4.75	1	4	2.5	6.75
16	1.25	1.5	1.5	1.5	1.5
17	4	3.25	4.75	3	6.25
18	4.25	5.25	6	4.25	6.5
19	5	1.25	1.5	1.5	1.5
20	2.5	4.25	3.25	3	3.5

APPENDIX M

Raw Data for Psychological Distress (PD)

PD

Subject #	Day1	Day 2	Day 3	Day 4	Day 5
1	1	1	1	1	1.25
2	1.75	3.75	2.5	2.75	2.25
3	1.25	3.5	1	1.5	1.5
4	1.75	3	2	1	1.75
5	2	1.5	1.75	1.75	2.25
6	1	1.5	1.25	1	1
7	1	1	1	1	1
8	1.75	1	4.25	1.5	1
9	3	1	1	1	1
10	1.25	1.25	2	2	2.25
11	1.75	3.25	3	1.75	2
12	1	1.25	1.25	2.5	2.25
13	1	1	1	1	1
14	2.25	2.75	3.5	3.25	4.75
15	1.25	1	2	1.5	4.75
16	1	1	1	1	1
17	2.25	1	2.75	1.25	5.25
18	1.75	1.5	1.5	1.25	1.5
19	1	1	1	1	1
20	2	4.75	2	2.25	2.75